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(54) Title: BUTENOLIDE DERIVATIVES AS ANTI–CANCER AGENTS

(57) Abstract

A new butenolide (furandione) anti–cancer agent is 3,4dichloro–5(1'–methyl–1'formylamino)–2(5H)furanone, which is synthesized by the reaction of an alkylformamide (e.g., N–methylformamide) and a halogenated acylchloride (e.g., dichloroacetylchloride). This compound interacts with nucleophilic sites within a cell. It has demonstrated profound anti–tumour activity against murine colon adenocarcinomos (MAC 13 and MAC 15A), M5076–recticulum cell sarcoma, and several normally chemoresistant human colon xenografts, SW620, HCT 116 and DLD–1.

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BUTENOLIDE DERIVATIVES AS ANTI-CANCER AGENTS

BACKGROUND OF THE INVENTION

Technical Field

The present invention relates generally to chemical compounds having anti-tumour effects and, more particularly, to new butenolides: 3,4 dichloro-5(1'-methyl-1'formylamino)-2(5H) furanone, 3,4 dichloro-5(1'-methyl-1'formylamino)-2(3H) furanone and derivatives thereof, which may be synthesized by the reaction of an alkylformamide (e.g., N-methylformamide) and a halogenated acylchloride (e.g., dichloroacetylchloride) or a di-halogenated carboxylic acid anhydride.

Description of the Related Art

Chlorinated compounds are potential immunomodulators in the management of both neoplastic and viral diseases. For example, it is known that certain halogenated carboxylic acids, such as trichloro- and dichloro-acetic acid, are capable of producing halogen that reacts with macromolecules within a cell such as protein. N-methylformamide is a known anti-cancer agent, but its use clinically as an anti-neoplastic agent has been limited because of associated hepatotoxicity.

It would be desirable to provide other novel compositions and treatment methods using chlorinated compounds.

BRIEF SUMMARY OF THE INVENTION

A principal objective of the present invention is to provide a novel chemical compound having anti-tumour effects.

Another object of the invention is to provide a butenolide (furandione) compound having anti-tumour effects that can be easily produced in large quantities

and that is water soluble.

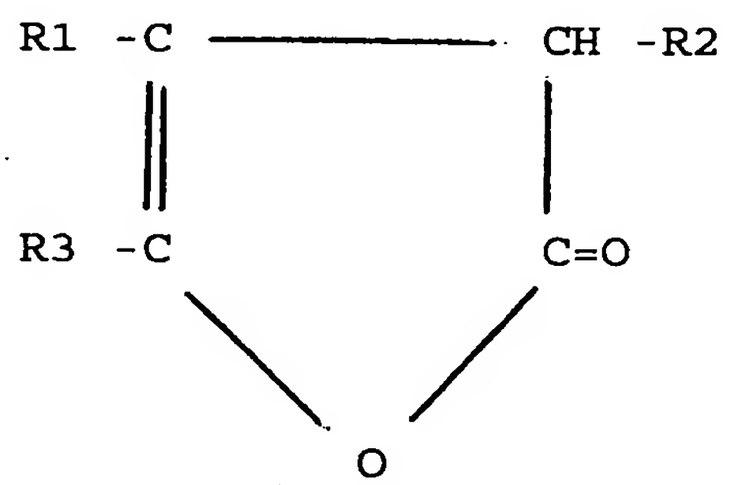
A further object of the present invention is to provide an anti-cancer agent that has a broad spectrum of tumour-reduction activity against syngeneic mouse tumours.

Yet a further object of the invention is to provide a novel compound that has anti-tumour activity against chemoresistant xenografts.

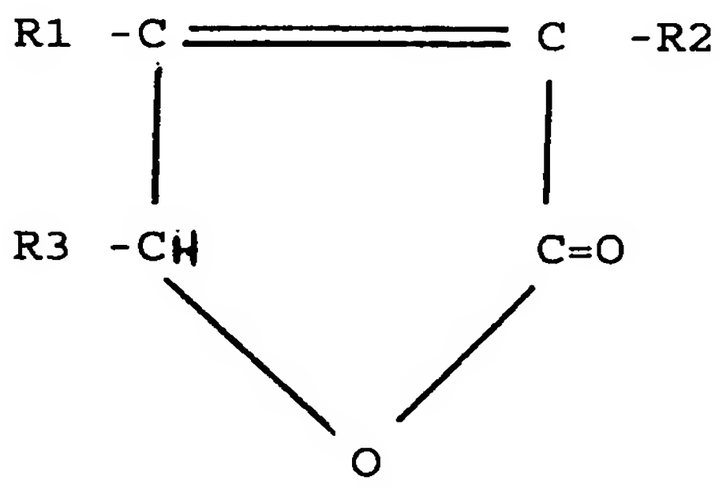
Still another object of this invention is to provide methods for treating tumours in mammals using such novel compounds.

These and other objects of the invention are accomplished by a butenolide (furandione) anti-cancer agent or compound of the formula as set out in the accompanying claims.

Thus, according to a first aspect, the present invention provides a compound of formula:



or its isomer:



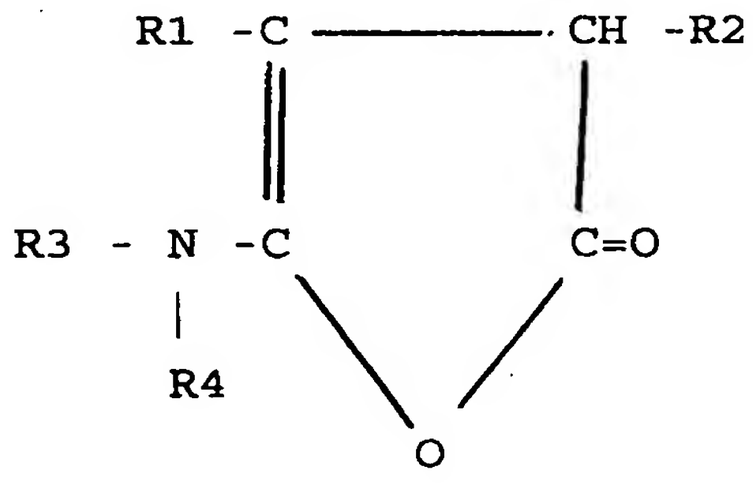
wherein

R1 and R2 are independently hydrogen, chlorine, bromine, fluorine, hydroxyl, amino, alkyl or aryl group or any functional group; and

R3 is independently alkyl, aryl, hydroxyl, or any functional group.

Preferably, R1 and/or R3 is  and

R4 and R5 are independently alkyl, aryl, hydroxyl, alkoxy or any functional group. In particular, a compound of the formula:



is provided, wherein R1 and R2 are independently hydrogen, chlorine, bromine, fluorine, hydroxyl, amino, alkyl or aryl group or any functional group and R3 and R4 are independently alkyl, aryl, hydroxyl, alkoxy or any other group (preferably functional). When each of R1 and R2 are chlorine, R3 is methyl, and R4 is formyl (-CHO), the compound is 3,4 dichloro -5(1'-methyl - 1'formylamino)-2(5H) furanone or is 3,4 dichloro -5(1'-methyl - 1'formylamino)-2(3H) furanone.

In the present invention, the term "functional group" is understood to include: a hydrogen atom, a halogen atom, alkyl, hydroxy, aryl, heteroaryl, alkoxy,

acyloxy, aryloxy, amino, alkylamino, arylamino, acyl, amide, imido, isocyanate, epoxide and vinyl group, and more complex heterocyclic groups such as hydantoin or rhodanine. Where R1 is amino it may be substituted by any of the above functional groups, or it may itself form part of one of the more complex R3 heterocyclic groups, thus forming a bicyclic system. Alkyl groups in any of the above, which of course include within their scope cyclo-alkyl groups, may be: replaced by (cyclo)alkenyl or (cyclo)akynyl; substituted by an oxo group or hydrazone thereof;

Other preferred compounds of the invention are:

a compound as claimed in the first aspect of the invention wherein one of R1 or R2 is chlorine, and the other is hydrogen;

a compound as claimed in the first aspect of the invention wherein one of R1 or R2 is chlorine, and the other is OH;

a compound as claimed in the first aspect of the invention wherein one of R1 or R2 is hydrogen, and the other is OH;

a compound as claimed in the first aspect of the invention wherein both R1 and R2 are hydrogen; and

a compound as claimed in the first aspect of the invention wherein both R1 and R2 are OH.

According to a second aspect, the present invention provides a composition comprising a compound, as set out in the first aspect of the invention, and a pharmaceutically acceptable carrier or diluent. Preferably the composition is a pharmaceutical composition.

One or more compounds as set out in the first aspect of the invention may be present in association with one or more non-toxic pharmaceutically and/or veterinarily acceptable carriers and/or diluents and/or adjuvants and if desired other active ingredients. Suitable carriers or diluents include water, buffers,

sodium bicarbonate, oil, polyethylene glycol (PEG), glycerol and liposomes, or any which are known in the art (e.g. *Handbook of Pharmaceutical Excipients* (1994) 2nd Edition, Eds. A. Wade/PJ Weller, The Pharmaceutical Press, American Pharmaceutical Association). The precise ingredients of a composition will vary depending on the use and nature of the composition; for example, if the composition is to be administered parenterally, adjuvants such as a local anaesthetic, preservative and buffering agents may be advantageously included.

The composition may be in the form suitable for oral, rectal, topical or parenteral administration. Suitable forms may be, for example, aqueous or oily suspensions, powders, granules, emulsions, solutions, cream, ointment, gels and liposomes.

All preferred features of the first aspect of the invention also apply to the second.

According to a third aspect, the present invention provides a method of making a compound as set out in the first aspect of the present invention, by reacting an alkylformamide and either a di-halogenated carboxylic anhydride or a halogenated acylchloride.

A general method of this aspect of the present invention involves reacting an alkylformamide with a halogenated acylchloride, such as dichloroacetyl-chloride, or a di-halogenated carboxylic acid anhydride, such as dichloroacetic anhydride, in a suitable solvent such as dichloromethane or chloroform. The reaction is cooled in an ice bath. Cold water is added with excess sodium bicarbonate powder and stirred for several hours. The bicarbonate powder is removed and the solvent evaporated and purified by column chromatography. The product is further evaporated and dried under vacuum.

The third aspect of the invention also covers a method of making a composition as set out according to the second aspect of the invention. This method comprises mixing together the compound (according to the

first aspect of the invention) and a carrier or diluent. The mixing can be carried out using techniques known in the art.

Preferred features of aspects one and two also apply to the third aspect of the invention.

According to a fourth aspect, the present invention provides a method of treating a tumor in a mammal which comprises administering to said mammal an amount of a compound, as set out in the first aspect of the present invention, effective to reduce the size of said tumor.

This aspect of the invention is particularly relevant to the treatment of humans, but is also applicable to general veterinery industry, in particular domestic pets such as dogs and cats and farm animals such as horses, pigs, cattle, sheep, etc.

Tumors treated with the compounds or compositions of the present invention may be solid tumors such as colon, ovary, or lymphomas etc, and/or other types of tumors such as leukaemias.

The particular dosage regime will ultimately be determined by the attending physician and will take into consideration such factors as the medication being used, animal type, age, weight, severity of symptoms and/or severity of treatment being or to be applied, method of administration of the medication, adverse reactions and/or other contraindications. Specific defined dosage ranges can be determined by standard designed clinical trials with patient progress and recovery being fully monitored. Such trials may comprise an escalating dose design using a low percentage of the maximum tolerated dose in animals as the starting dose in man. Preliminary guidance for dosage ranges can be taken from the results given in the experimental section of this text and by the following ranges which have been extrapolated to humans.

The medication according to this aspect of the invention may be given to a patient in combination with

other suitable treatment, such as radiotherapy and/or surgical procedures.

Preferred features of aspects one to three also apply to the fourth aspect of the invention.

According to a fifth aspect, the present invention provides a compound or a composition as set out in the first or the second aspect of the invention for use in medicine.

All relevant features of the first to fourth aspects of the invention also apply to the fifth aspect.

According to a sixth aspect, the present invention provides a compound or a composition as set out in the first or the second aspect of the invention for use in the treatment of a tumor, preferably to reduce the size of the tumor.

All relevant features of the first to fifth aspects of the invention also apply to the sixth aspect.

According to a seventh aspect, the present invention provides the use of a compound as set out in the first aspect of the invention in the manufacture of a medicament for the treatment of a tumor.

All relevant features of the first to sixth aspects of the invention also apply to the seventh aspect.

In one particular preferred embodiment, this novel butenolide is synthesized by the reaction of N-methylformamide with dichloroacetylchloride. It has been found that this compound interacts with nucleophilic sites within a cell and has effective antitumour activity against, e.g., murine colon adenocarcinomas (MAC13 and MAC15A), M5076-recticulum cell sarcoma, and human colon xenografts, SW620, HCT116 and DLD-1 (which are normally chemoresistant).

Generalizing, the present invention is directed toward a novel composition preferably formed by reacting an alkylformamide and an halogenated acylchloride or anhydride to generate the novel butenolide (furandione) compound, and use of such compounds as anti-cancer

agents. Compounds of the present invention may be water- or lipid-soluble.

In the formulae of the present invention, alkyl groups include a chain length of 1 to 20 carbon atoms, preferably 1 to 6 carbon atoms.

BRIEF DESCRIPTION OF THE DRAWINGS

For a more complete understanding of the present invention and the advantages thereof, reference should be made to the following Detailed Description taken in connection with the accompanying drawings in which:

FIGURE 1 is a diagram illustrating the formation of a preferred butenolide composition in accordance with the invention;

FIGURE 2 is diagram illustrating the mechanism by which the preferred butenolide composition is believed to react with nucleophilic sites in cells to effect the purposes of the present invention;

FIGURE 3 is a table showing anti-tumor activity of the butenolide compound against M5 tumours;

FIGURE 4 is a table showing anti-tumor activity of the butenolide against MAC 13 tumours;

FIGURE 5 is a table showing anti-tumor activity of the butenolide composition and 5-Fluorouracil ("5FU") against MAC 15 tumours;

FIGURE 6 is a table showing anti-tumor activity of the butenolide composition against MAC 15 tumours;

FIGURE 7 is a table showing anti-tumor activity of the inventive composition and 5FU against MAC 15 tumours;

FIGURE 8 is a table showing anti-tumor activity of the inventive composition against MAC 15A tumours with the drug being dissolved in 0.1% sodium bicarbonate;

FIGURE 9 is a table showing anti-tumor activity of the inventive composition against MAC 15 tumours with the drug being dissolved in 0.2% sodium bicarbonate;

FIGURE 10 is a table showing anti-tumor activity of

the inventive composition against MAC 15 tumours with the drug being dissolved in water;

FIGURE 11 is a table showing anti-tumor activity of the preferred butenolide composition and several variations thereof against MAC 13 tumours;

FIGURE 12 is a table showing anti-tumor activity of several test compositions against MS tumours;

FIGURE 13 is a chart showing anti-tumor activity of the inventive composition against SW620 xenografts;

FIGURE 14 is a chart showing anti-tumor activity of inventive composition against HCT116 xenografts;

FIGURE 15 is a chart showing anti-tumor activity of the inventive composition against DLD-1 xenografts;

FIGURE 16 shows a route by which butenolides may be made, the form in which it may exist in equilibrium, as well as some example compounds;

FIGURE 17 shows examples of compounds which can be made by altering the side chain in order to manipulate lipophilicity;

FIGURE 18 shows how the biological activity of compounds maybe manipulated and studied by varying the halogen in the 3,4-position;

FIGURE 19 shows a sketch of how butenolide building blocks maybe prepared, starting with furfural;

FIGURE 20 shows further reactions which provide some other example compounds; and

FIGURE 21 shows how structure-activity relationships of the compounds of the present invention may be investigated.

FIGURE 22 shows a scheme for the synthesis of some example compounds of the present invention.

FIGURE 23 is a chart showing the results of treating MAC 15A tumor sc transplants with WOA081/96B, 5-fluorouracil and N-methylformamide.

FIGURE 24 shows the reaction of WOA081/96B with nitrophenylhydrazine. This reaction may be used for the structural investigation of the compounds of the present

invention, and is an example of a reaction of a compound of the present invention with a nucleophilic substance.

FIGURE 25 is a flow-chart showing a general process of producing a compound of the present invention.

FIGURES 26 to 33 show further reaction schemes for preparing compound according to the invention, and the compounds produced thereby.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention relates to a preferred butenolide (furandione) composition (sometimes referred to herein for convenience as "WOA 081/96B") and its anti-tumour activity against tumour lines with different histology, growth characters and spectra of chemosensitivity to standard agents. Several variations of this butenolide composition are also described as set forth below and are provided with different reference identifiers for convenience of discussion.

By way of brief background, the mouse adenocarcinoma of the colon ("MAC") series of transplantable tumours has been used in a variety of chemotherapy studies. The MAC tumours are similar to human colon cancer. It has also been demonstrated that the spectrum of chemotherapeutic sensitivity shows good correlation with the response rates of standard chemotherapeutic agents against colorectal cancers. The anti-tumour activity is usually close to maximum tolerated dose indicating the general insensitivity of the tumour system. Similarly, human colon tumour xenografts SW620, HCT116 and DLD-1 are chemoresistant, particularly the slow growing DLD-1 tumour. The M5076 is a reticulum cell sarcoma and is generally chemosensitive.

Preparation of WOA 081/96B

FIGURE 1 illustrates a method of preparing WOA 081/96B in accordance with the present invention. WOA 081/96B is prepared preferably by the reaction between equimolar amounts of dichloroacetylchloride or dichloroacetic anhydride with dry N-methylformamide in 500 mls of dry dichloromethane. The reaction preferably is carried out in the following steps. The dry N-methylformamide is dissolved in dry dichloromethane (or in any other suitable solvent, e.g., chloroform). The dichloroacetylchloride or dichloroacetic anhydride in dichloromethane is added dropwise with stirring while the solution is cooled in an ice-bath maintaining reaction temperature between 60-80°C. Use reflux condenser and stir for 6-24h. Cold water is added, and the solution is stirred for another 1 hour. Add in small portions excess sodium bicarbonate powder and stir overnight (or for a similar time period).

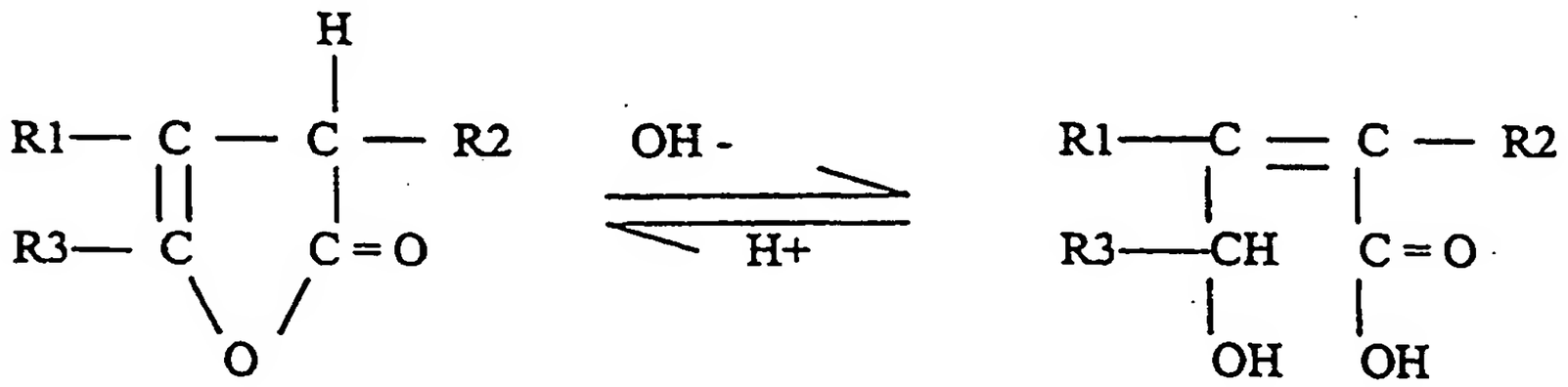
The excess bicarbonate powder is removed by a filtering process using a sintered glass funnel with a vacuum pump. The dichloromethane is evaporated, leaving a yellow or yellow-orange residue. This is purified by column chromatography using petroleum ether:dichloromethane:ethanol (2:2:1) or petroleum ether:diethyl ether (1:1). The purity of the product is checked on a silica TLC plate using petroleum ether:diethyl ether (1:1) as solvent. The solvent is evaporated and the product dried under vacuum overnight (or for such a time period) with magnesium sulphate or calcium chloride. The resulting product is stored below 4°C and protected from both light and moisture.

Spectroscopic and elemental analysis of the 2-nitrophenylhydrazone derivative suggest the formation of WOA 081/96B.

The above-described method is useful to prepare

other derivatives of halogenated acylchloride or halogenated carboxylic acid anhydride with N-methylformamide or any other amides. The present invention includes such resulting derivatives. Thus, for example, compositions referred to herein as WOA 080/96 and WOA 082/96 are products formed from the reaction between trichloro- and chloroacetyl chloride with N-methylformamide, respectively. These two products as will be seen also demonstrate significant anti-tumour activity, thereby further validating the efficacy of the inventive technique.

The compounds of the present invention may assume different equilibrium forms depending on the pH of the medium. Without being bound, the following equation is proposed to be a possible equilibrium which occurs for a compound of the present invention:



The scope of the invention is understood to include all such alternative forms of the compounds of the present invention.

Example

Test Protocol

Pure strain NMRI and BDF_1 (age 6-8 weeks) from an inbred colony were used for transplanting MAC and M5076 tumours, respectively. They were fed on an RM3E diet (Lillco-England) and water ad libitum.

MAC 13 and MAC 16 tumours were transplanted into mice by sc implantation of tumour fragments (about 1 x 2

mm) in the flank. MAC 15A ascites tumours were transplanted in male mice by sc inoculation of 1 x 10^6 tumour cells in 0.2 ml of physiological saline.

An evaluation of WOA 081/96B against SW620, HCT116 and DLD-1 human colon tumour xenografts was carried out by independent testing. The results are shown in FIGURES 13-15. In the independent testing, the following protocol was devised and used.

Tumour fragments excised from donor animals were first transplanted subcutaneously in the flank of the male NCR-nude mice.

WOA 081/96B was diluted either in PBS or 1% sodium bicarbonate solution and treatment was by a once daily intraperitoneal (ip), subcutaneous (sc), peroral (po) or intravenous (iv) administration.

Each group contained a minimum of 5 tumour-bearing mice. With the more rapidly growing MAC13, MAC15A and M5076 sc tumours, chemotherapy commenced 2 days after implantation. MAC13 and MAC15A sc tumours are palpable at this stage and anti-tumour responses were assessed about 14 days later by recording tumour weights. With the slower growing MAC16, SW620, HCT1 16 and DLD-1 tumours, chemotherapy did not conunence until tumours could be reliably measured. Tumour volume was calculated from the formula $a^2 \times b/2$, where a is the smaller diameter and b is the larger.

Statistical significance was determined by either Mann-Whitney U test or a computerised method (statview 512+).

Experimental Results

The WOA 081/96B compound interacts with nucleophilic sites within a cell as shown in FIGURE 2 and was thus tested for anti-tumour activity.

As shown in FIGURES 3-15, WOA 081/96B has significant activity against MAC1-3, MAC15A, M5076 and human colon tumour xenografts SW620, HCT116 and DLD-1.

It was active when given daily or on alternate days independent of the route of administration.

FIGURE 3 is a table showing anti-tumor activity of WOA 081/96B against M5 tumours transplanted sc. The treatment was given ip daily, with the drug being dissolved in water. 8 animals per group, sacrificed on day 17 after transplant.

FIGURE 4 is a table showing anti-tumor activity of WOA 08 1/96B against MAC 13 tumours 14 days after sc transplants. 5 animals per group. The drug was dissolved in water and given ip.

FIGURE 5 is a table showing anti-tumor activity of WOA 081/96B and 5FU against MAC 15 tumours. The tumours were transplanted sc and treatment given ip daily. The drugs were dissolved in water.

FIGURE 6 is a table showing anti-tumor activity of WOA 081/96B against MAC15 tumours transplanted sc. Treatment was provided using various routes. The drug was dissolved in water. 5 animals per group.

FIGURE 7 is a table showing anti-tumor activity of WOA 081/96B and 5FU against MAC 15 tumours transplanted sc. The treatment was given ip and the drugs were dissolved in water. 6 animals per group.

FIGURE 8 is a table showing anti-tumor activity of WOA 081/96B against MAC15A tumours transplanted sc. Treatment was given either ip or orally. The drug was dissolved in 0.1% sodium bicarbonate and administered using various schedules. 5 animals per group.

FIGURE 9 is a table showing anti-tumor activity of WOA 081/96B against MAC 15 tumours transplanted sc. Treatment was given ip. The drug was dissolved in 0.2% sodium bicarbonate and Vitamin E dissolved in olive oil. 5 animals per group.

FIGURE 10 is a table showing anti-tumor activity of WOA 081/96B against MAC15 tumours transplanted sc. Treatment was given ip, either daily or on alternate days. The drug was dissolved in water. 5 animals per

group.

FIGURE 11 is a table showing anti-tumor activity of WOA 080/96, 081/96B and 082/96 against MAC13 tumours 14 days after sc transplant. The drugs were dissolved in water and given ip on days 1,2,3,4,5 and 8. 5 animals per group.

FIGURE 12 is a table showing anti-tumor activity of WOA 080/96, 081/96B, 082/96, 083/96, and 084/96 against MS tumours 20 days after sc transplant. The drugs were dissolved in water and administered ip. 5 animals per group.

FIGURE 13 is a chart showing anti-tumor activity of WOA 081/96B against SW620 xenografts.

FIGURE 14 is a chart showing anti-tumor activity of WOA 081/96B against HCT116 xenografts.

FIGURE 15 is a chart showing anti-tumor activity of WOA 081/96B against DLD-1 xenografts.

FIGURE 20 shows further reactions which provide some other example compounds. The reaction of mucochloric acid and hydrazine will provide diaza lactones and the reaction with isocyanates will furnish carbamates which are closer to the original lead structure. A wide variety of isocyanates or isothiocyanates are commercially available to synthesize a series of analog compounds. Umpolung in the 5-position: numerous N nucleophiles which are either prepared by using thionyl chloride or DEAD will be connected to the 5-position.

FIGURE 23 is a chart showing the results of treating MAC 15A tumor sc transplants with WOA081/96B, 5-fluorouracil and N-methylformamide.

As the results show, this compound demonstrates significant anti-tumour activity over a wide range of chemoresistant tumours.

Dose-limiting body weight loss is preventable by formulating it in 1% sodium bicarbonate solution.

FIGURES 26 to 33 show further reaction schemes for

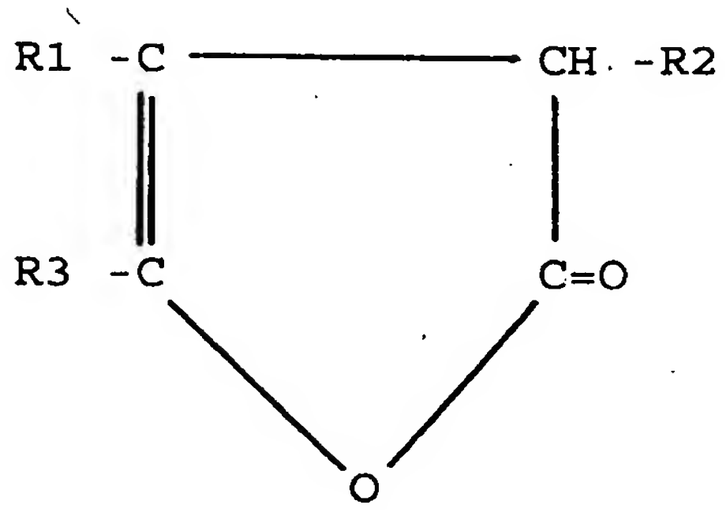
preparing compound according to the invention, and the compounds produced thereby.

Having thus described our invention, what we claim as new and desire to secure by Letters Patent is set forth in the following claims.

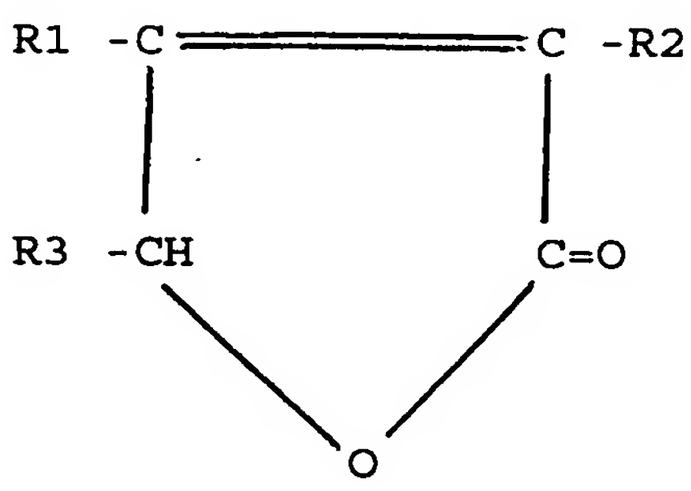
Other variations and modifications of this invention will be apparent to those skilled in the art alter careful study of this application. This invention is not to be limited except as set forth in the following claims. All references disclosed herein are incorporated by reference.

CLAIMS

1. A compound of formula:



or its isomer:



wherein

R1 and R2 are independently hydrogen, chlorine, bromine, fluorine, hydroxyl, amino, alkyl or aryl group or any functional group; and

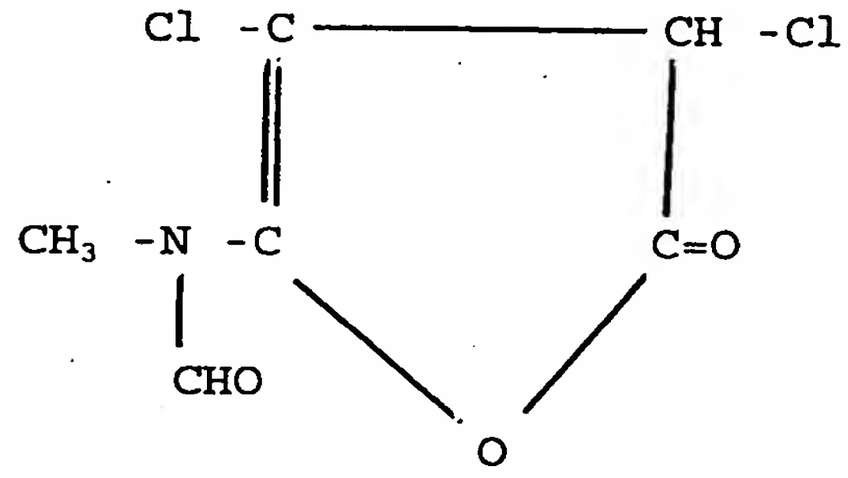
R3 is independently alkyl, aryl, hydroxyl, or any functional group.

2. A compound as claimed in claim 1 wherein R3 is N(R4)(R5) and R4 and R5 are independently alkyl, aryl, hydroxyl, alkoxy or any functional group.

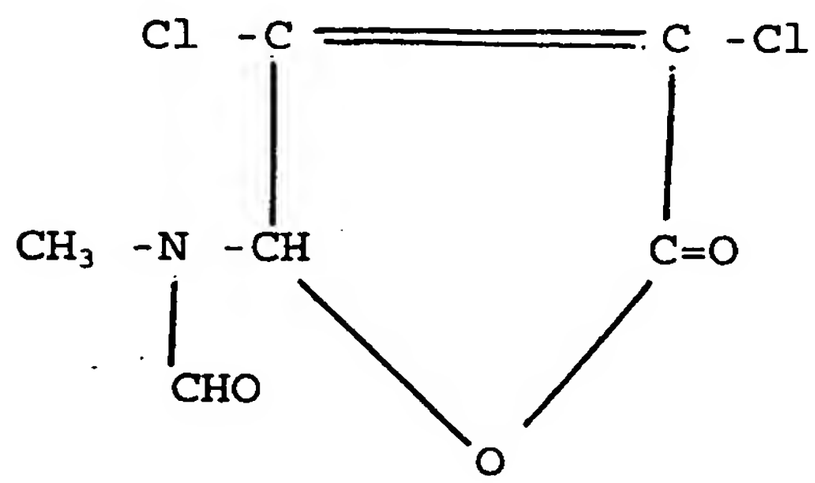
3. The compound of claim 1 or claim 2 wherein R1 and R2 are each chlorine and R3 is methyl.

4. The compound of claim 3 being:
3,4 dichloro -5(1'-methyl - 1'formylamino)-2(5H)furanone; or
3,4 dichloro -5(1'-methyl - 1'formylamino)-2(3H)furanone.

5. A compound of any of claims 1 to 3, being:



or



6. The compound of claim 4 or claim 5 synthesized by reacting N-methylformamide with dichloroacetylchloride.

7. The compound of claim 4 or claim 5 synthesized by reacting N-methylformamide with dichloroacetic anhydride.

8. The compound of any one of claims 1 to 5 synthesized by reacting an alkylformamide and a halogenated acylchloride.

9. The compound of any one of claims 1 to 5 synthesized by reacting an alkylformamide and a di-halogenated carboxylic acid anhydride.

10. A composition comprising a compound of any one of claims 1 to 9 and a pharmaceutically acceptable carrier or diluent.

11. A composition as claimed in claim 10 which is a pharmaceutical composition.

12. A method of making a compound as claimed in any one of claims 1 to 5 by reacting an alkylformamide and either a di-halogenated carboxylic acid anhydride or a halogenated acylchloride.

13. The method as described in claim 12 wherein the alkylformamide is N-methylformamide and the halogenated acylchloride is dichloroacetylchloride.

14. A method for treating a tumor in a mammal which comprises administering to said mammal an amount of a compound of any one of claims 1 to 9 effective to reduce the size of said tumor.

15. A compound as claimed in any one of claims 1 to 9, or a composition as claimed in claim 10 or claim 11, for use in medicine.

16. The use of a compound, as claimed in any one of claims 1 to 9, in the manufacture of a medicament for the treatment of a tumor.

PROPOSED MECHANISM OF FORMATION OF WOA 081/96B

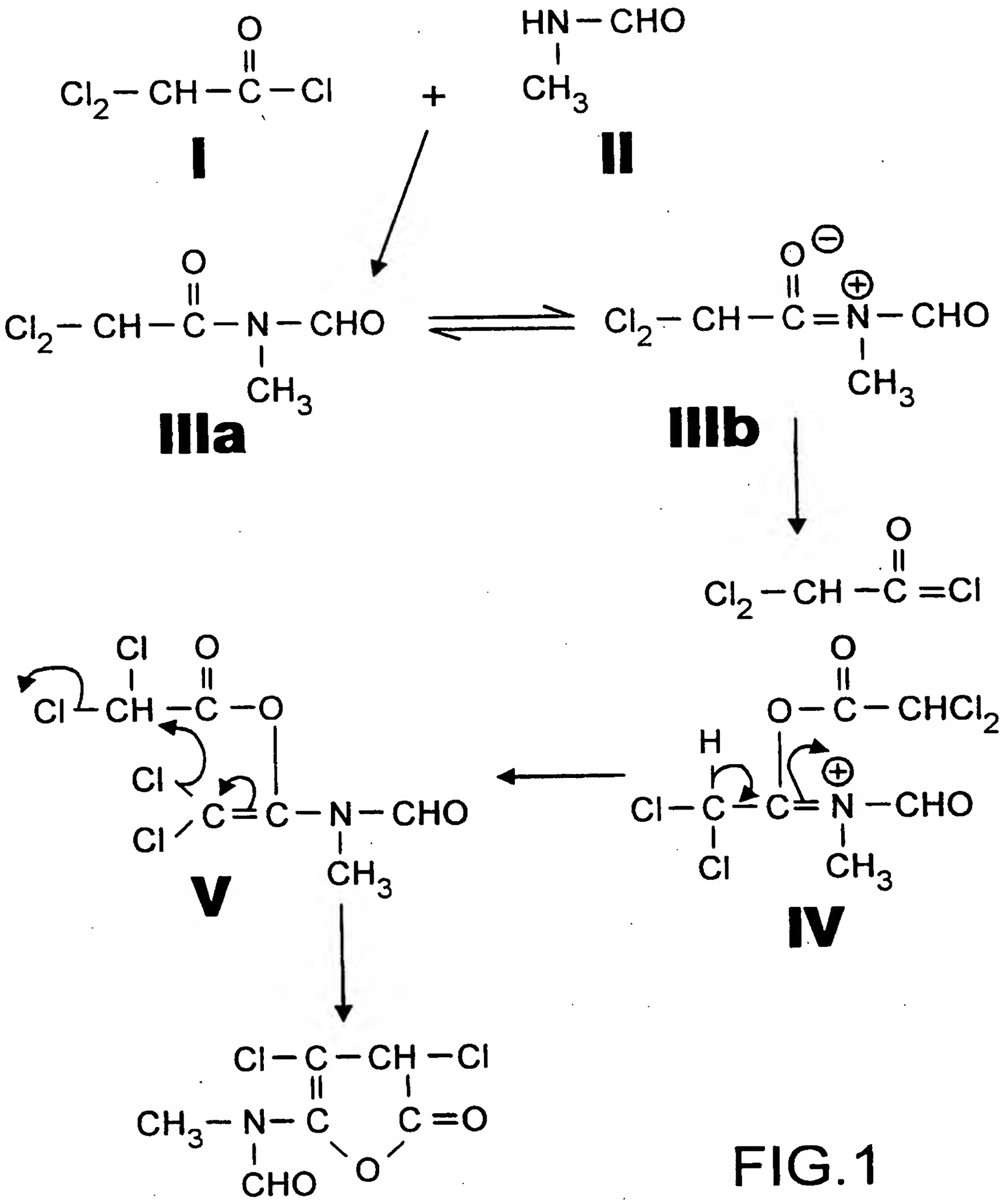


FIG.1

POTENTIAL REACTIONS OF WOA 081/96B IN CELLS

REACTION WITH NUCLEOPHILES (ṄUCH)

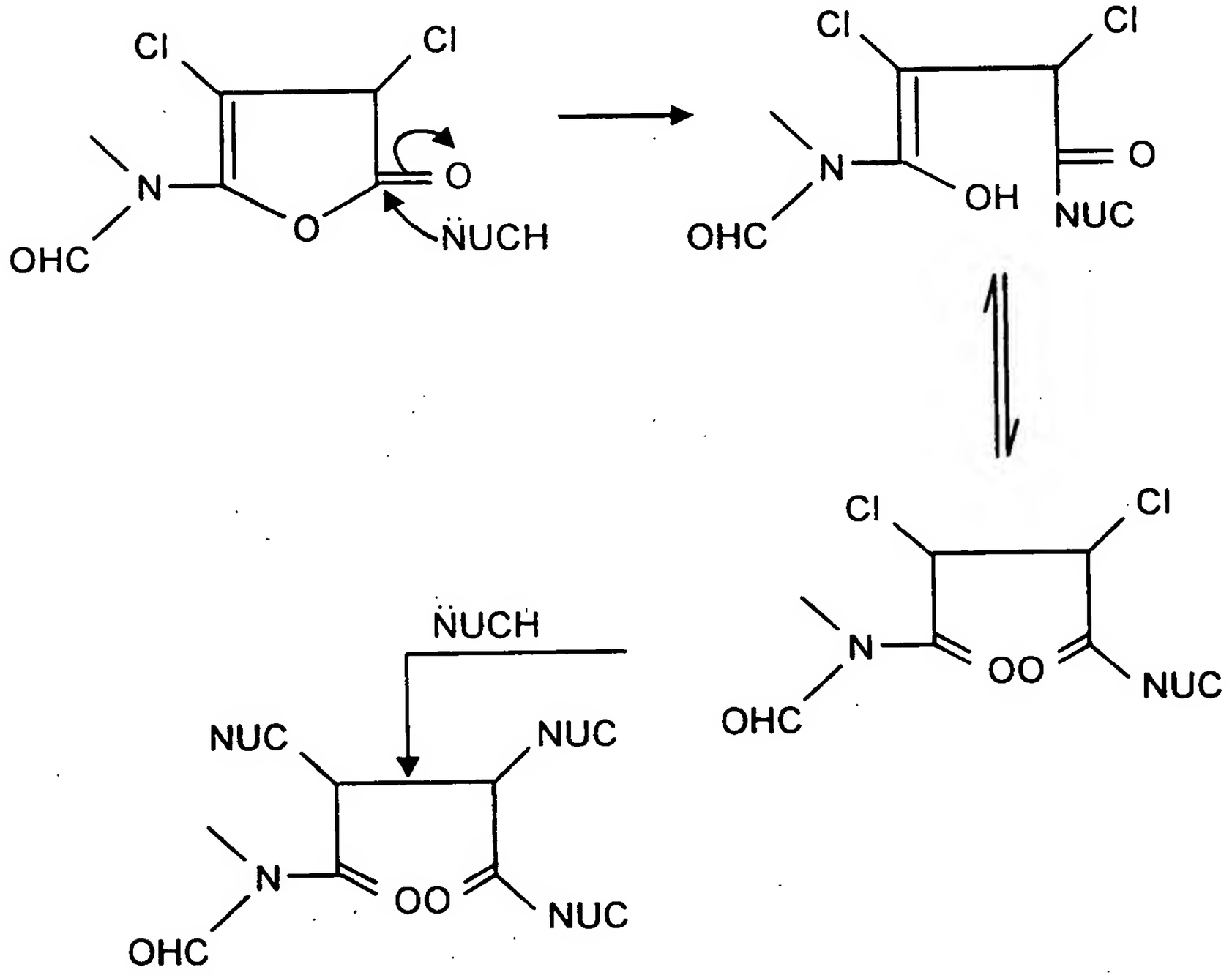


FIG.2

Compound Dose (mg/kgbwt)	Tumour Weight (g)	Mean Tumour Weight (g)	sem	p-value	% Tumour Weight inhibition
1600	0.0, 0.0, 0.0, 0.0, 0.1, 0.0, 0.0, 0.0	0.0125	0.0125	0.0008	99
1400	0.0, 0.0, 0.0, 0.1, 0.0, 0.0, 0.1, 0.1	0.0375	0.0183	0.001	96
1200	0.0, 0.0, 0.1, 0.1, 0.0, 0.0, 0.1, 0.0	0.0375	0.0183	0.001	96
1000	0.2, 0.2, 0.4, 0.0, 0.0, 0.4, 0.1, 0.1	0.0175	0.0559	0.002	82
800	0.2, 0.0, 0.0, 0.2, 0.3, 0.1, 0.4, 0.4	0.2	0.0567	0.003	79
600	0.6, 0.1, 0.8, 0.2, 0.1, 0.7, 0.2, 0.6	0.4125	0.1025	0.02	58
Control	0.9, 1.6, 0.6, 0.7, 0.2, 1.2, 1.3, 1.3	0.975	0.1623	-	-

FIG. 3

Dose (mg/kgbwt)	Days of treatment	Tumour Weight(g)	Means Tumour Weight(g)	sem	p-Value	Weight difference of animal (g)	% Tumour Weight Inhibition
1800	1-13	0.00, 0.00, 0.06, 0.08, 0.02, 0.00	0.03	0.01	0.0002	+0.8	96
1600	1-13	0.02, 0.02, 0.03, 0.04, 0.00, 0.06, 0.07, 0.02	0.03	0.008	0.0001	+7.3	96
1400	1-13	0.00, 0.00, 0.02, 0.08, 0.04, 0.09, 0.02, 0.08	0.04	0.01	0.0001	+9.1	94
1200	1-13	0.02, 0.15, 0.04, 0.07, 0.03, 0.08, 0.06, 0.07	0.07	0.01	0.0001	+6.4	90
1000	1-13	0.11, 0.07, 0.08, 0.09, 0.02, 0.02, 0.03, 0.02	0.06	0.01	0.0001	+7.3	92
Control		0.61, 0.70, 0.86, 0.46, 0.55, 0.71, 0.91, 0.71	0.69			+6.0	

FIG.4

Dose of (mg/kgbwt)	No.of animals	Days of sacrifice	Range	Median	Mean	T/Cx100	Difference in weight of animal
1800	8	8, 11, 15, 15, 15, 15, 20, 21	8-21	15	15	353	+0.7g
1600	8	8, 9, 10 10, 10, 11, 11, 11	8-11	10	10	235	+2.0g
1400	8	8, 8, 9, 9, 10, 10, 10, 10	8-10	10	9	218	+0.6g
1200	8	8, 8, 8, 9, 10 10, 10, 10	8-10	9	9	203	+4.1g
5FU 50	8	4, 4, 4, 4, 5, 5, 5, 5	4-5	5	5	109	+0.8g
Control	8	4, 4, 4, 4, 4, 4, 5, 5	4-5	5	5	100	+1.6g

FIG.5

Compound Dose (mg/kgbwt)	Route of admin	Tumour weight (g)	Mean Tumour weight (g)	sem	p-value	% Tumour weight inhibition	Diff. in animal weight	Days of treatment
1800	I.P	0.077, 0.167, 0.112, 0.140, 0.087	0.116	0.01	0.06	77	+0.3	1, 2, 3, 4, 5, 6
1600	I.P	0.077, 0.041, 0.083, 0.107,	0.079	0.01	0.04	85	+1.2	1, 2, 3, 4, 5, 6
1400	I.P	0.051, 0.072, 0.105, 0.119	0.087	0.01	0.09	83	0	1, 2, 3, 4, 5, 6
1800	Orally	0.177, 0.085, 0.185, 0.108, 0.178	0.146	0.02	0.05	71	+1.2	1, 2, 3, 4, 5, 6
1600	Orally	0.115, 0.087, 0.120, 0.035, 0.075	0.086	0.01	0.04	83	+1	1, 2, 3, 4,
1400	Orally	0.126, 0.163, 0.157, 0.136, 0.093	0.135	0.01	0.06	73	+0.3	1, 2, 3, 4,
1800	S.C	0.064, 0.072, 0.151, 0.100, 0.078	0.093	0.01	0.03	85	+0.4	1, 2, 3, 4, 6
1600	S.C	0.063, 0.096, 0.137, 0.103, 0.097	0.099	0.01	0.04	80	+1.2	1, 2, 3, 4, 6
1400	S.C	0.131, 0.091, 0.105, 0.095, 0.094	0.103	0.07	0.05	80	+2	1, 2, 3, 4, 6
Control (not treated)		0.378, 0.206, 1.072, 0.420, 0.446	0.504	0.14	-	-	+1.3	-

FIG.6

Dose (mg/kgbwt)	Days of treatment	Tumour Weight(g)	Means Tumour Weight(g)	sem	p-Value	Difference in animal weight (g)	% Tumour Weight Inhibition
081/96 B 1600	1-5	0.084, 0.047, 0.076, 0.092, 0.107, 0.066,	0.078	0.01	0.01	+0.1	79
081/96B 1400	1-5	0.270, 0.189, 0.206, 0.087, 0.096, 0.163	0.168	0.03	0.02	+1.2	55
081/96B 1200	1-5	0.118, 0.090, 0.187, 0.086, 0.118, 0.110	0.118	0.01	0.02	+0.6	50
081/96B 1000	1-5	0.091, 0.083, 0.115, 0.119, 0.108, 0.084	0.100	0.06	0.02	+1.8	73
5FU 50	1-5	0.063, 0.078, 0.122, 0.158, 0.084, 0.125	0.105	0.01	0.02	-0.6	72
Control	1-5	0.522, 0.505, 0.219, 0.264, 0.145, 0.612	0.377	0.08		+3.0	0

FIG.7

Dose mg/kgbwt	Route of admin	Schedule and days of treatment	Tumour weight(g)		Mean tumour weight(g)	sem	p-value	% Weight tumour inhibition	Diff.in animal weight
600	I.P	Daily 1-6	0.074, 0.064, 0.066	0.057, 0.034,	0.059	0.006	0.006	84	+0.4
600	I.P	Alt. days 1, 3, 5	0.140, 0.032, 0.072	0.046, 0.046,	0.067	0.019	0.01	82	+1.6
300	I.P	x2 daily	0.080, 0.150, 0.198	0.084, 0.162,	0.135	0.022	0.04	64	+0.5
200	I.P	x3 daily	0.074, 0.056, 0.097	0.062, 0.100,	0.078	0.008	0.01	79	+1.4
600	Orally	Daily 1-6	0.146, 0.132, 0.091	0.114, 0.102,	0.117	0.01	0.01	69	+1.0
600	Orally	Alt. days 1, 3, 5	0.228, 0.217, 0.162	0.104, 0.098,	0.162	0.03	0.01	57	+1.3
300	Orally	x2 daily	0.128, 0.087, 0.124	0.098, 0.090,	0.085	0.09	0.01	77	+0.5
Control	Not Treated		0.428, 0.547, 0.324	0.407, 0.157,	0.373	0.06	0.01	-	+1.4

FIG. 8

Compounds Dose: (mg/kgbwt)	Days treated	Tumour Weight	Means Tumour Weight	sem	p-Value	Mean weight difference of animal	% Weight tumour Inhibition
WOA086/96B 600	1-7	0.091, 0.152 0.113, 0.211, 0.267	0.167	0.032	0.0001	+1.3	85
WOA086/96B 600 + Vit E 100	1-7	0.079, 0.156, 0.179, 0.102 0.157	0.136	0.018	0.0001	0	88
Vit E 100	1-7	0.962, 0.712, 1.251, 1.355, 1.076	1.071	0.113	0.575	0	5
Control (untreated)		0.876, 1.265, 1.104, 1.107, 1.272	1.125	0.072	-	+1.2	0

FIG.9

Comp. WOA081/96B Dose mg/kgbwt	Route of admin	Days treated	Tumour weight(g)		Mean tumour weight(g)	sem	p-value	% Weight tumour inhibition	Mean Diff. in animal weight
1000	I.P	1, 3, 5	0.090, 0.065, 0.061	0.071, 0.071,	0.072	0.005	0.003	83	-0.4
800	I.P	1, 3, 5	0.093, 0.187, 0.067	0.061, 0.113,	0.104	0.022	0.001	76	-0.2
600	I.P	1, 3, 5	0.059, 0.049, 0.014	0.053, 0.050,	0.045	0.007	0.001	90	+0.4
1000	I.P	1 - 5	0.088, 0.108, 0.052	0.092, 0.044,	0.077	0.012	0.001	82	-0.2
800	I.P	1 - 5	0.134, 0.271, 0.052	0.084, 0.071,	0.122	0.032	0.02	72	-0.5
600	I.P	1 - 5	0.114, 0.054, 0.080	0.052, 0.098,	0.080	0.012	0.004	82	+0.5
Control (untreated)	-	-	0.466, 0.321,	0.519, 0.426,	0.435	0.042	-	0	+3.5

FIG. 10

Compound (WOA)	Dose (%V/V) (200ul given)	Tumour weight(g)	Mean Tumour weight(g)	sem	p values	% Tumour weight inhibition
080/96	8	0.07, 0.04, 0.08, 0.11, 0.07	0.07	0.01	0.02	80
	4	0.07, 0.13, 0.11, 0.05, 0.23	0.03	0.03	0.03	66
	2	0.01, 0.09, 0.06, 0.23, 0.26	0.13	0.05	0.08	63
*081/96B	8	0.0, 0.03, 0.0, 0.03, 0.08	0.03	0.02	0.01	91
	4	0.11, 0.13, 0.08, 0.07, 0.09	0.10	0.01	0.01	71
	2	0.04, 0.09, 0.0, 0.1, 0.11	0.07	0.02	0.01	80
082/96	8	0.10, 0.11, 0.10, 0.0, 0.08	0.08	0.02	0.01	77
	4	0.08, 0.01, 0.23, 0.15, 0.16	0.13	0.04	0.09	63
	2	0.13, 0.25, 0.19, 0.29, 0.11	0.19	0.03	0.06	46
Control		0.35, 0.61, 0.25, 0.29, 0.26	0.35	0.07	-	0

FIG.11

Compound (WOA)	Dose (%v/v) (200ul)	Days of treatment	Tumour weight(g)	Mean tumour weight(g)	scm	p value	% Tumour weight inhibition
080/96	10	1,4,9,11, 13	0.12, 0.07, 0.18, 0.13, 0.10	0.12	0.01	0.002	86
	8	1,4,9,11, 13,15	0.16, 0.10 0.04, 0.14, 0.17	0.12	0.02	0.008	86
081/96B	10	1,4,9,11, 13	0.14, 0.06 0.0, 0.0	0.05	0.03	0.005	94
	8	1,4,9,13,	0.0, 0.0, 0.06, 0.0, 0.0	0.01	0.01	0.001	99
082/96	10	1,2,3,4,9, 11,13,15	.2, 0.0, .16, .16, .18	0.14	0.04	0.005	84
	8	1,2,3,4,9, 11,13,15	.27, .13,.12, .11	0.16	0.04	0.005	81
083/96 (same as 080/96)	10	1,2,3,4,9, 11,13,15	.15, .19, .43, .35 .08	0.24	0.06	0.01	72
	8	1,2,3,4,9, 11,13,15	0.0, .04, .09, .27, .23	0.13	0.05	0.002	85
084/96 (same as 081/96B)	10	1,9,13	0.0, 0.0, .07, 0.0, .1	0.03	0.02	0.001	97
	8	1,9,11, 15	0.0, .02, .06, .07, 0.0	0.03	0.02	0.001	97
Control			1.14, 0.58, 0.72, 0.89, 0.96	0.86	0.09		0

FIG.12

SW620 XENOGRAFTS TREATED WITH WOA 081/96B

TREATMENT: 0.8 g/Kgbwt DAYS 0-11

1.6 g/Kgbwt DAYS 0-5

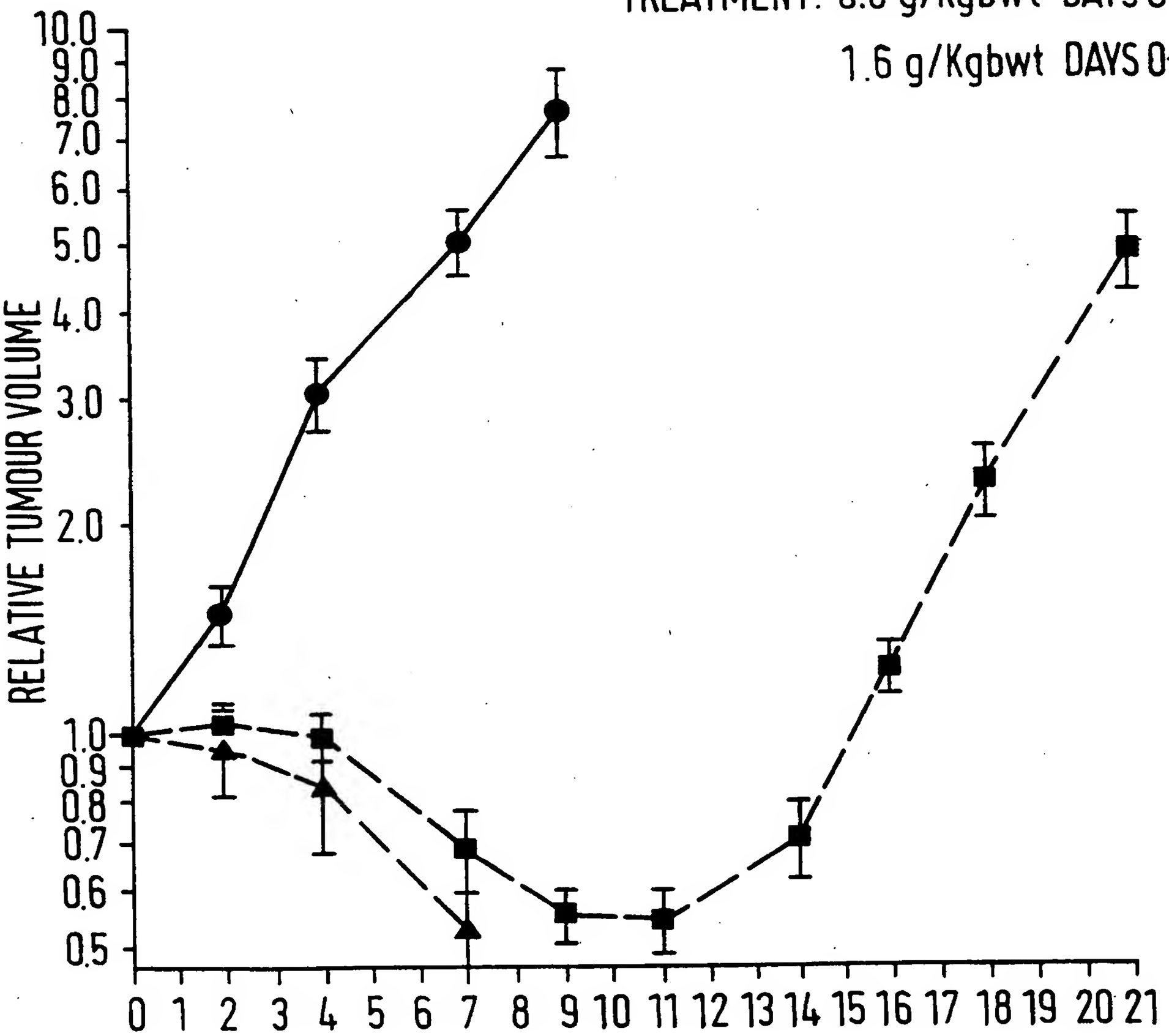


FIG. 13

HCT116 XENOGRAFTS TREATED WITH WOA 081/96B

TREATMENT: DAYS 0-5

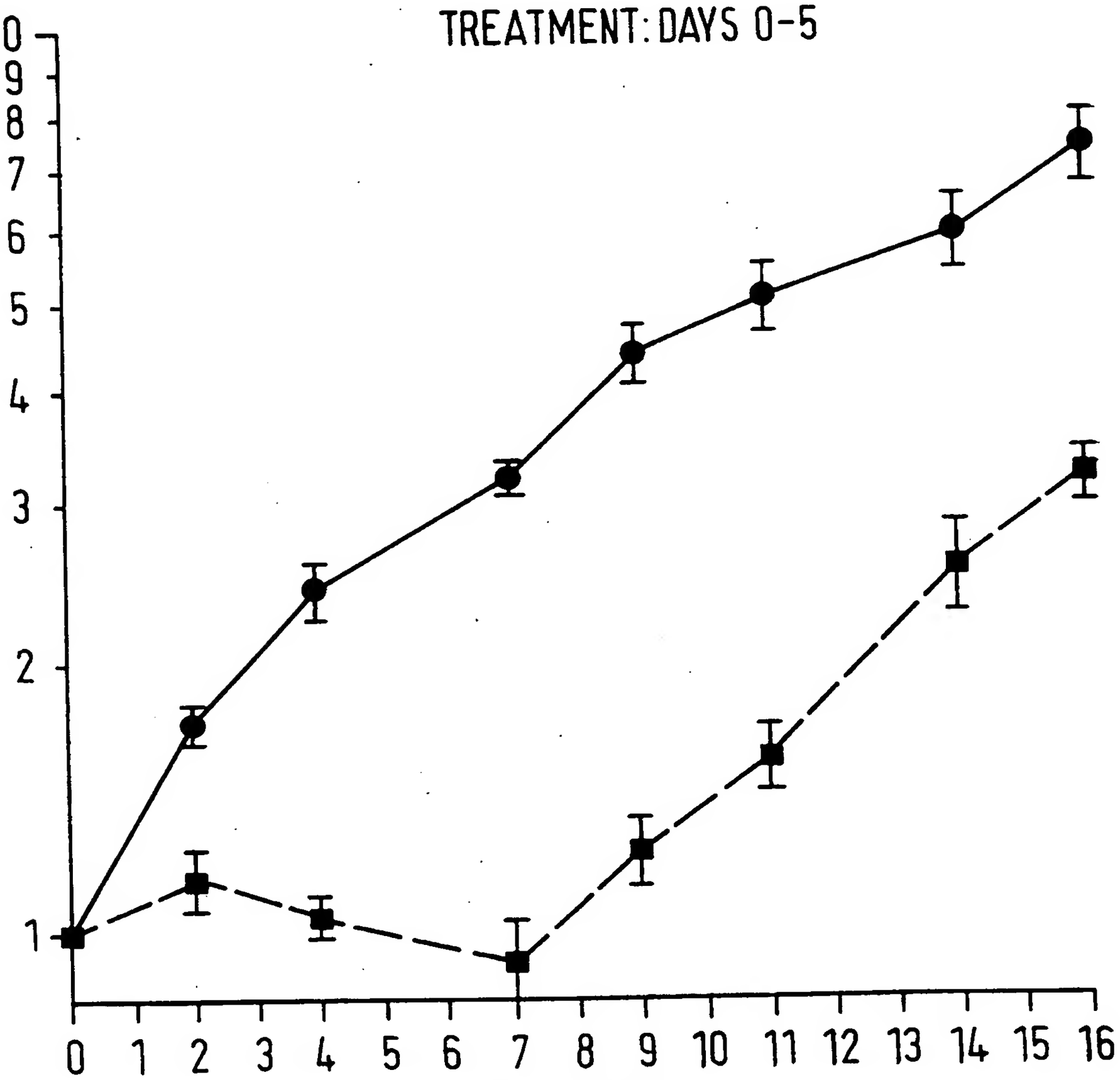


FIG. 14

DLD-1 XENOGRAFTS TREATED WITH WOA 081/96B

TREATMENT: DAYS 0-7

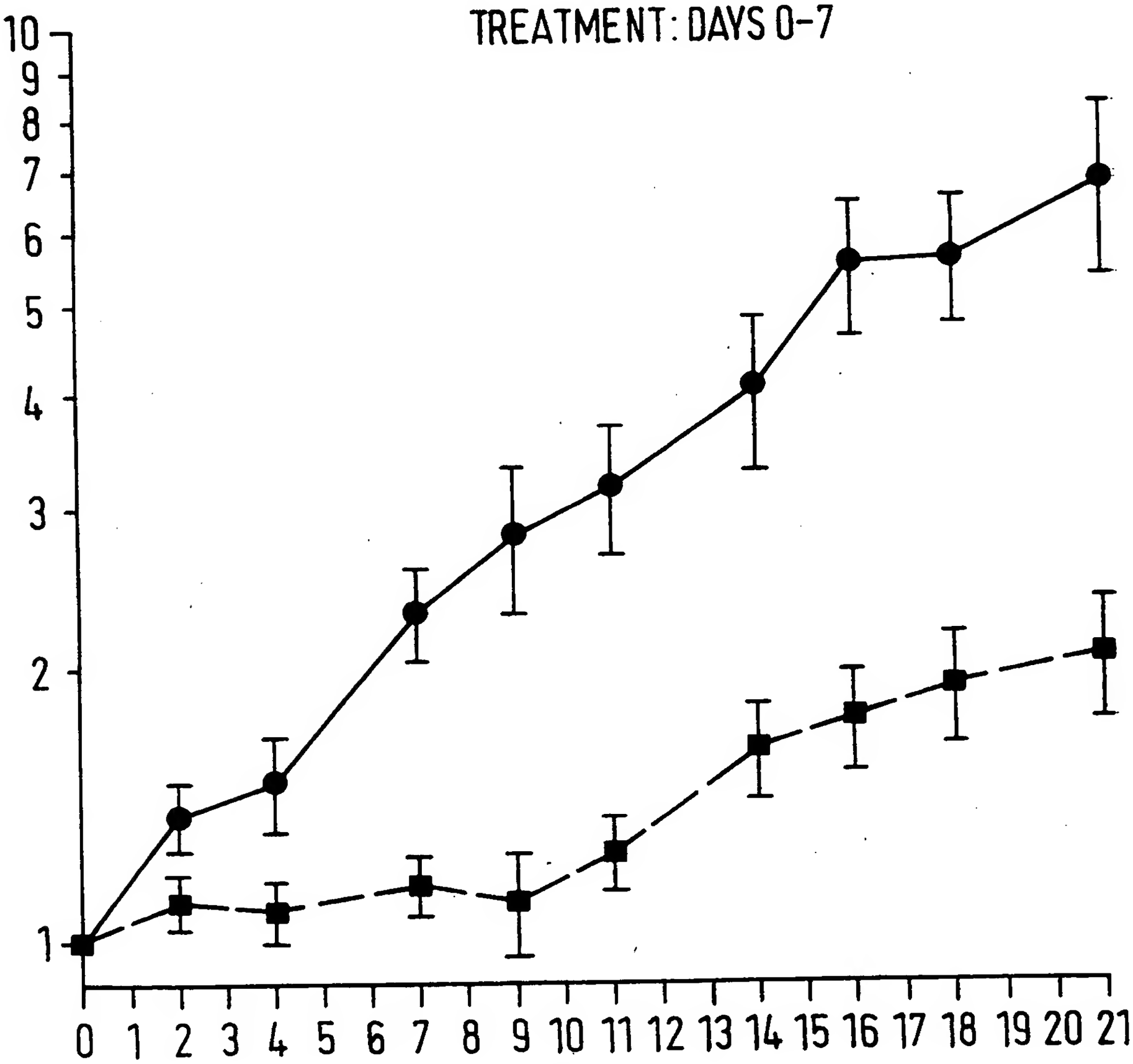
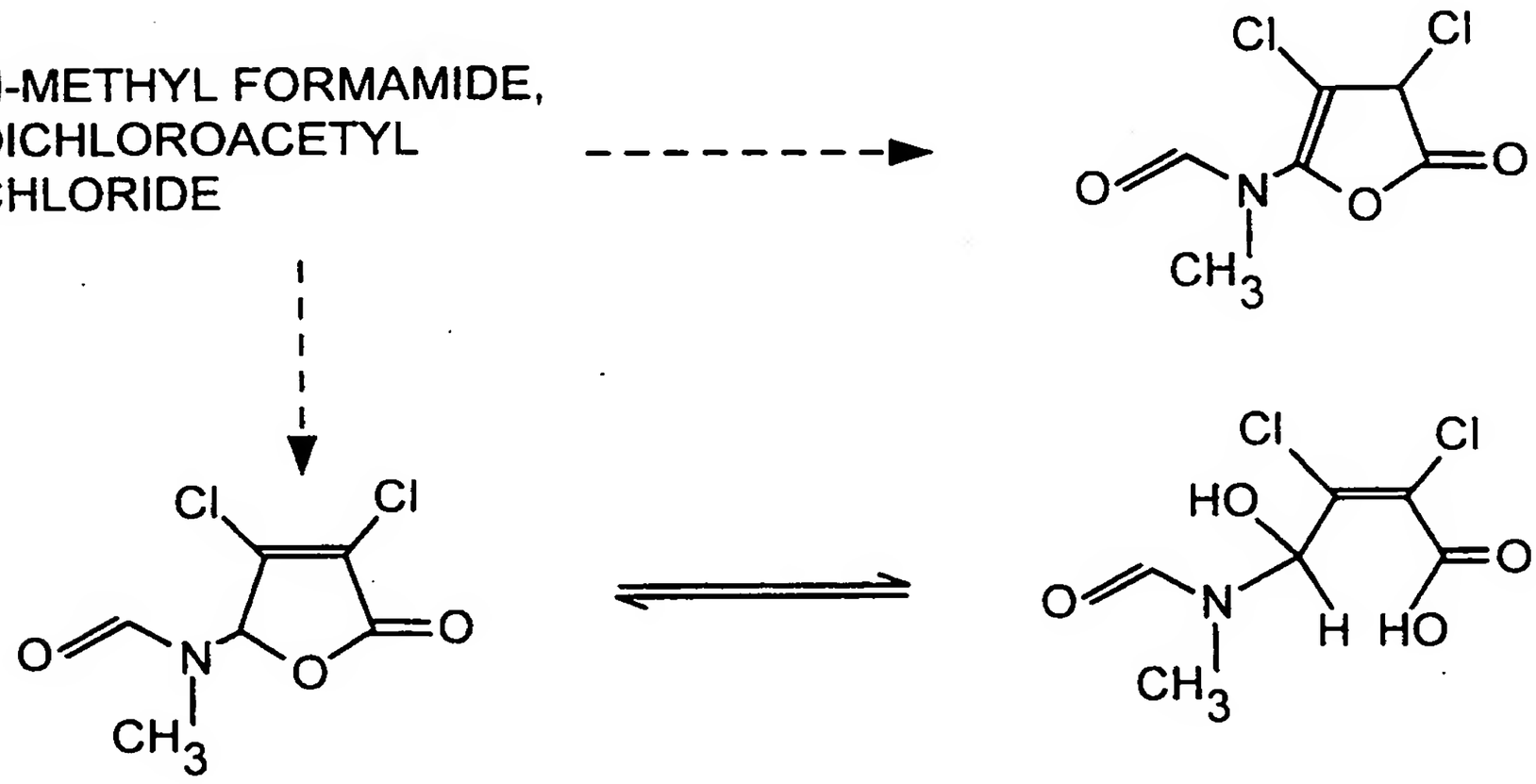


FIG. 15

BUTENOLIDES AS NOVEL ANTITUMOUR AGENTS:

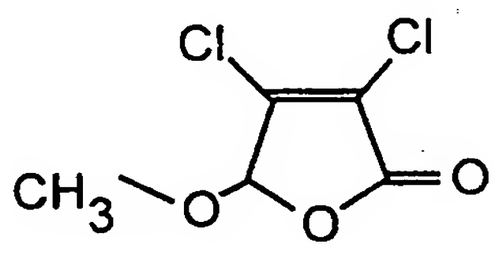
INITIAL STUDIES:

N-METHYL FORMAMIDE,
DICHLOROACETYL
CHLORIDE



1. PURIFICATION
2. ISOLATION

SOME BUTENOLIDE COMPOUNDS:



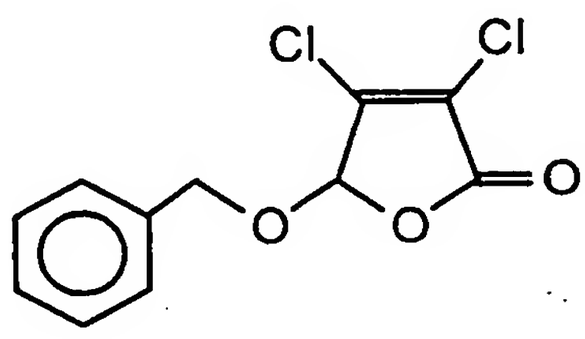


FIG.16

Side chain, manipulation of lipophilicity:

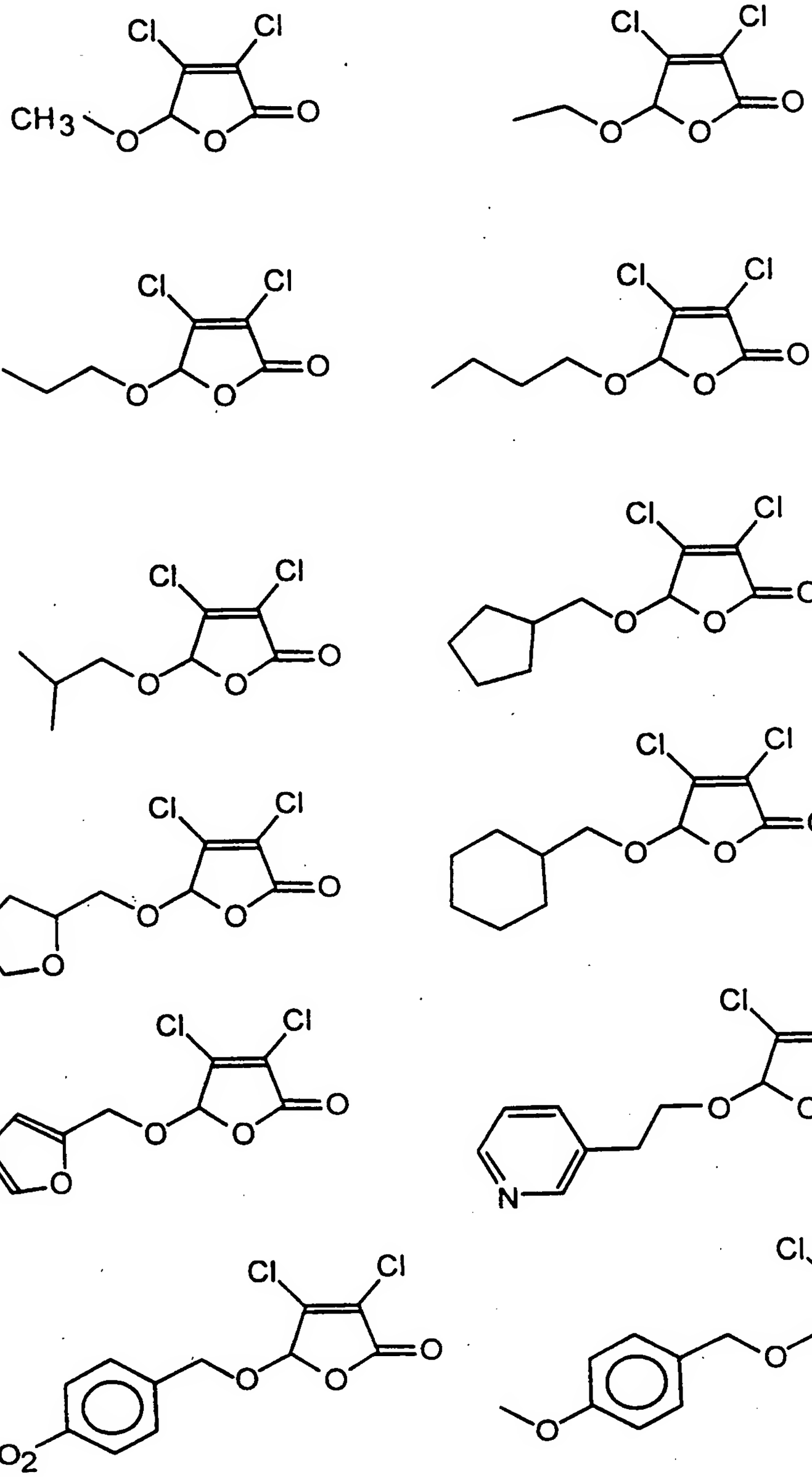


FIG. 17

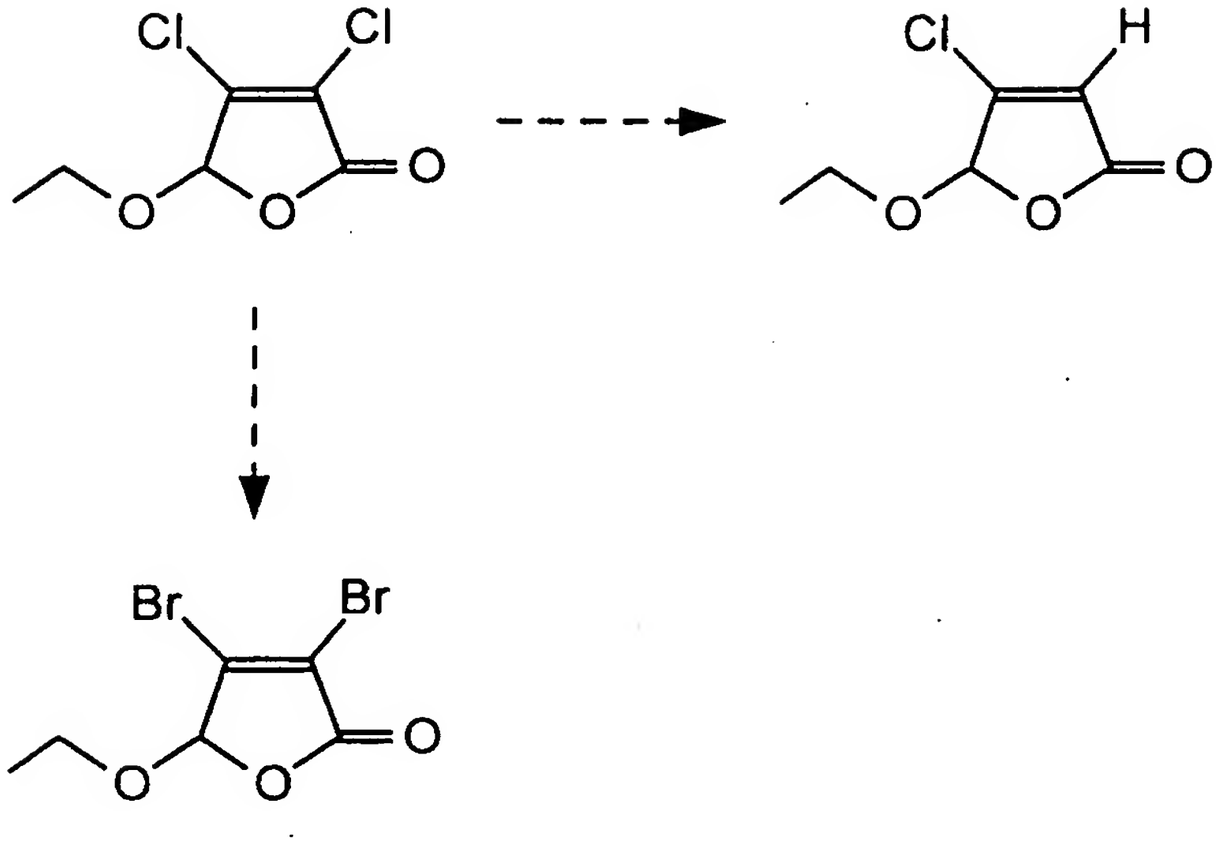


FIG.18

PREPARATION OF BUTENOLIDE BUILDING BLOCKS:

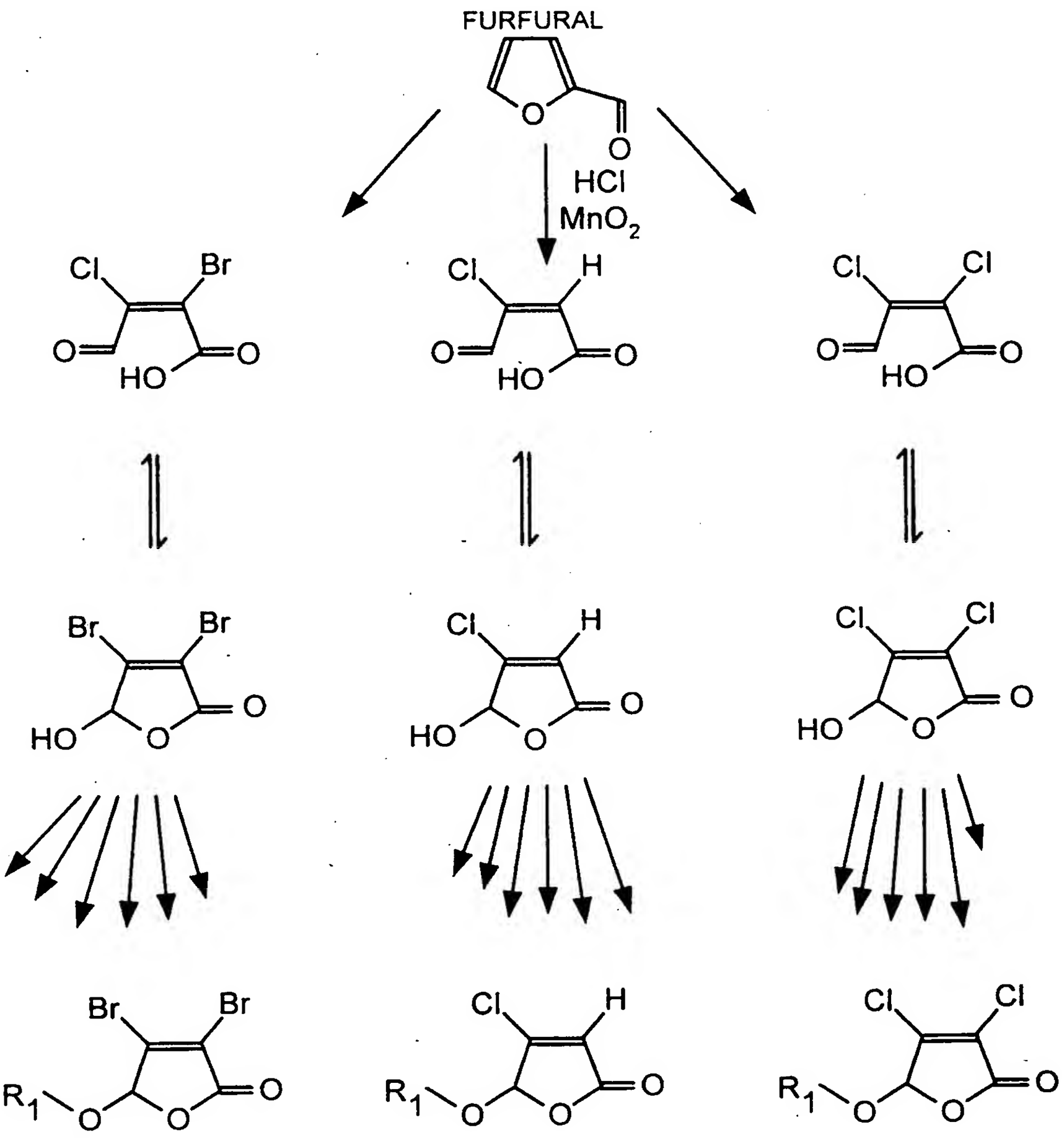


FIG.19

SYNTHESIS OF O, N-ACETALES AND CARBAMATES:

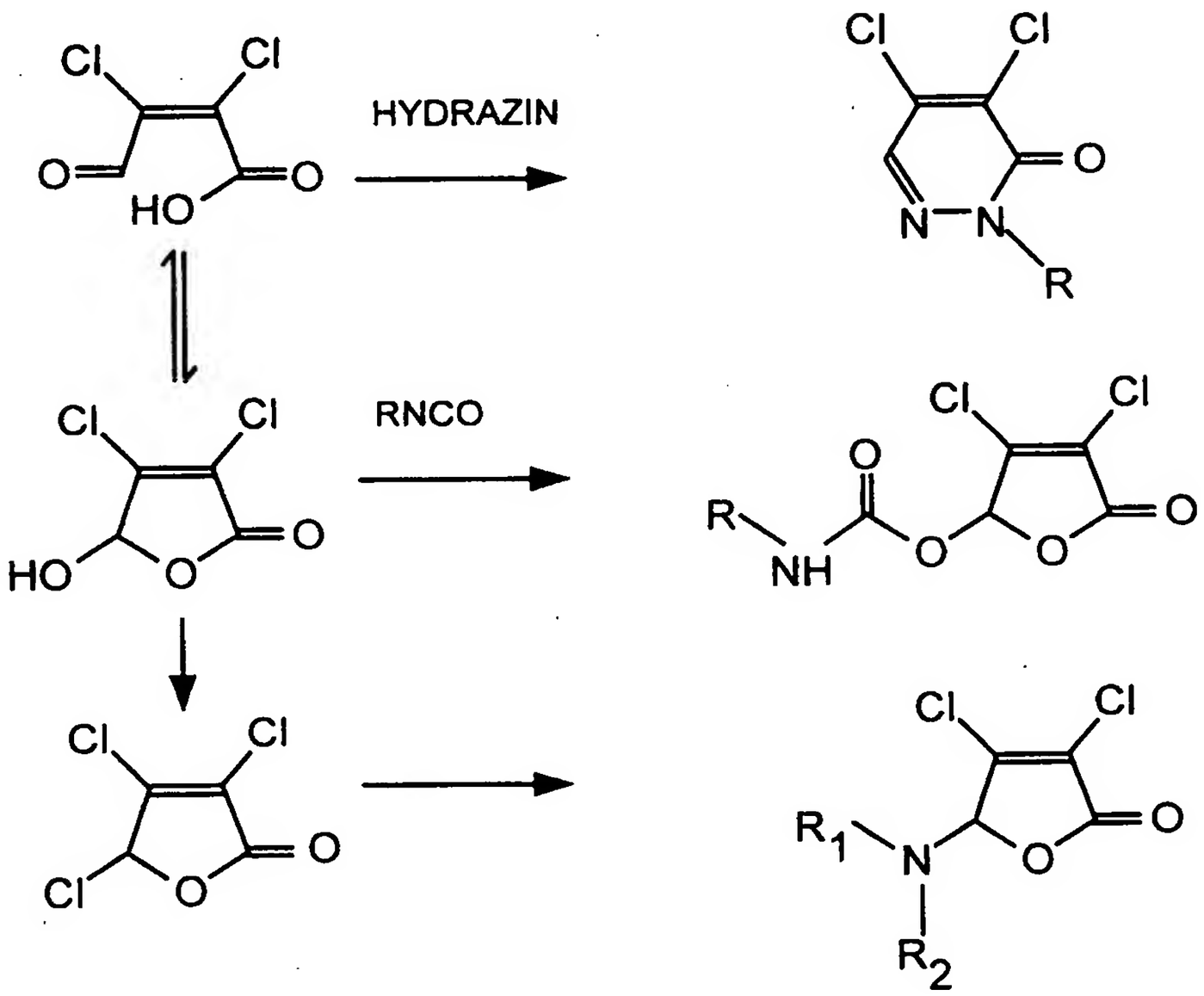
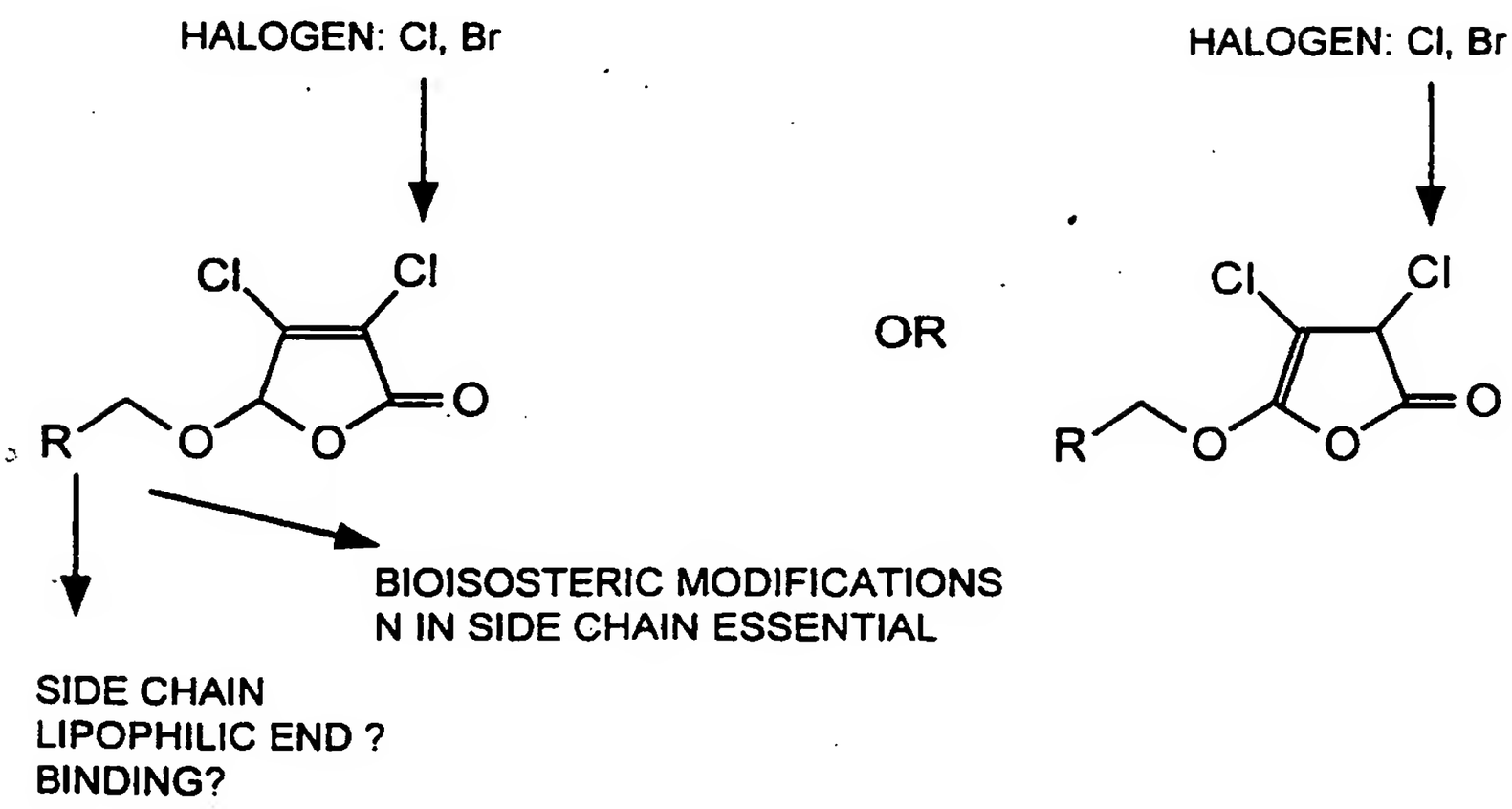


FIG.20

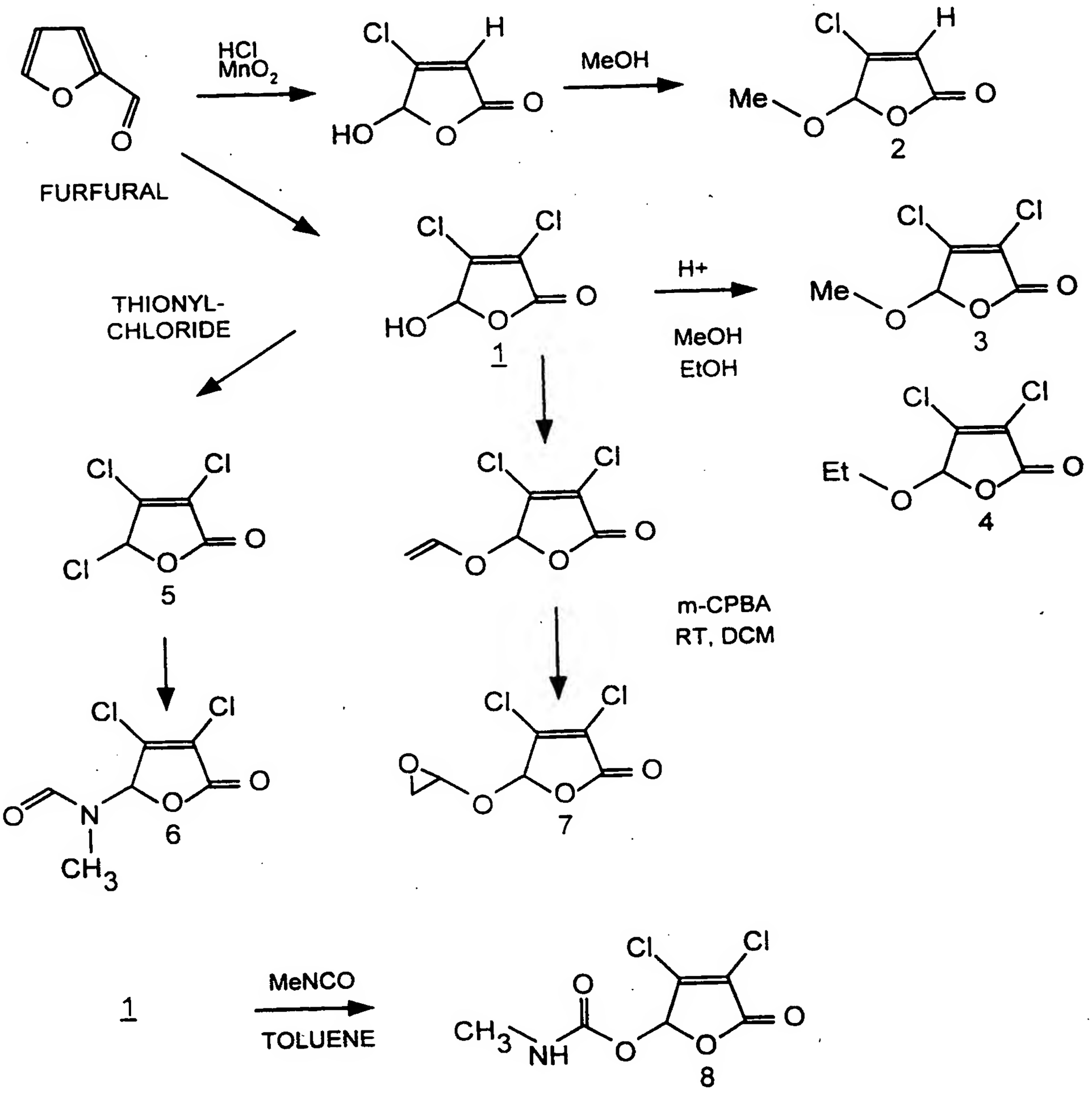
STRUCTURE ACTIVITY RELATIONSHIPS



- INFLUENCE OF SIDE CHAIN, LIPOPHILIC END, BINDING
- BIOLOGICAL ACTIVITY, BROMINE OR CHLORINE, IS CHLORINE IN 3-POSITION REQUIRED FOR ANTITUMOUR ACTION?
- IS POTENCY ENHANCED BY INTRODUCING N IN THE SIDE CHAIN (CARBAMATES) ?

FIG.21

THE SCHEME FOR THE SYNTHESIS OF SOME EXAMPLE COMPOUNDS:



MeOH	-METHANOL
EtOH	-ETHANOL
m-CPBA	-META-CHLOROPEROXYBENZOIC ACID
RT	-ROOM TEMPERATURE
DCM	-DICHLOROMETHANE
MeNCO	-METHYL ISOCYANATE

FIG.22

MAC15A TUMOUR SC TRANSPLANT TREATED WITH WOA081/96B, 5FU AND NMF IP. 5 ANIMALS PER GROUP

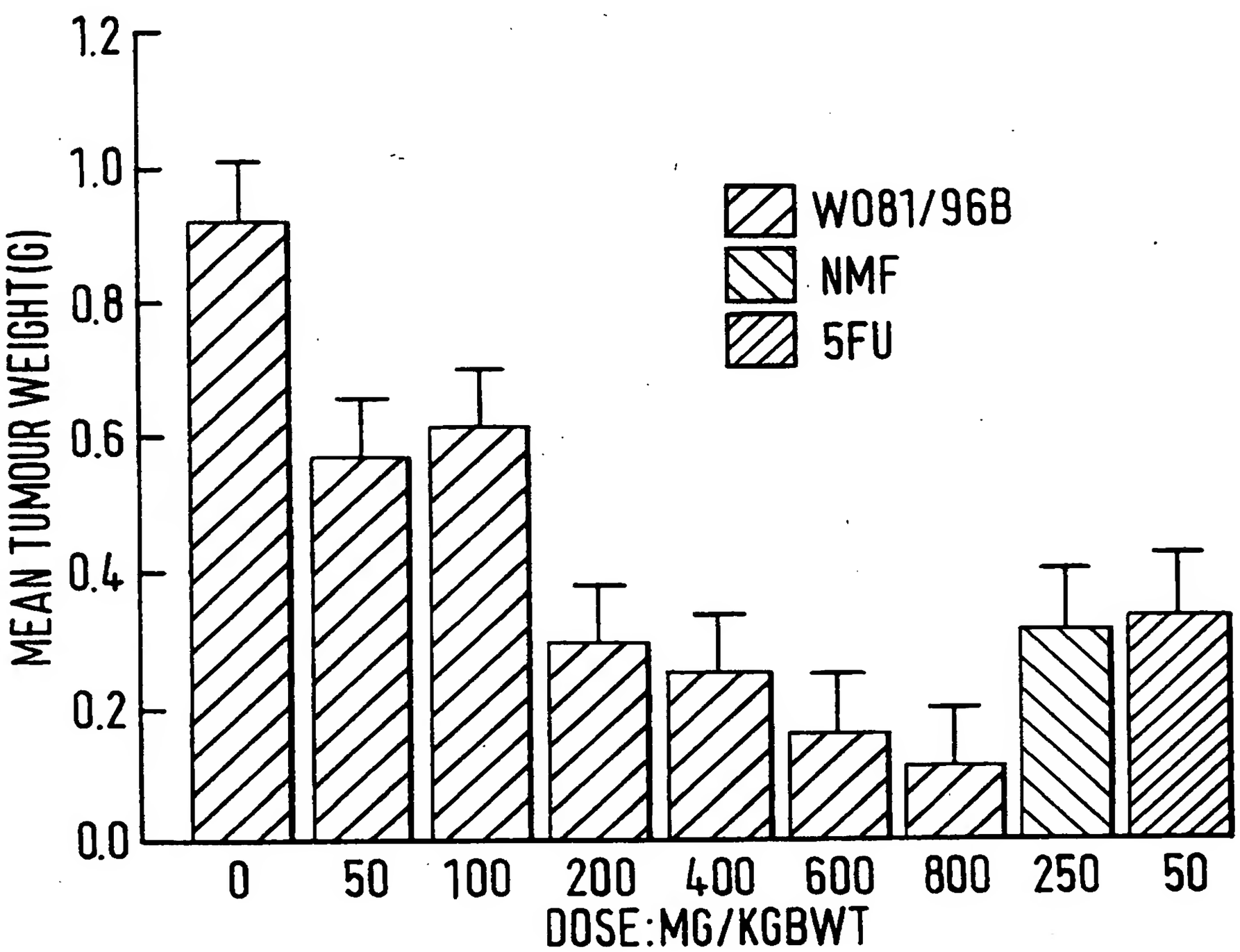
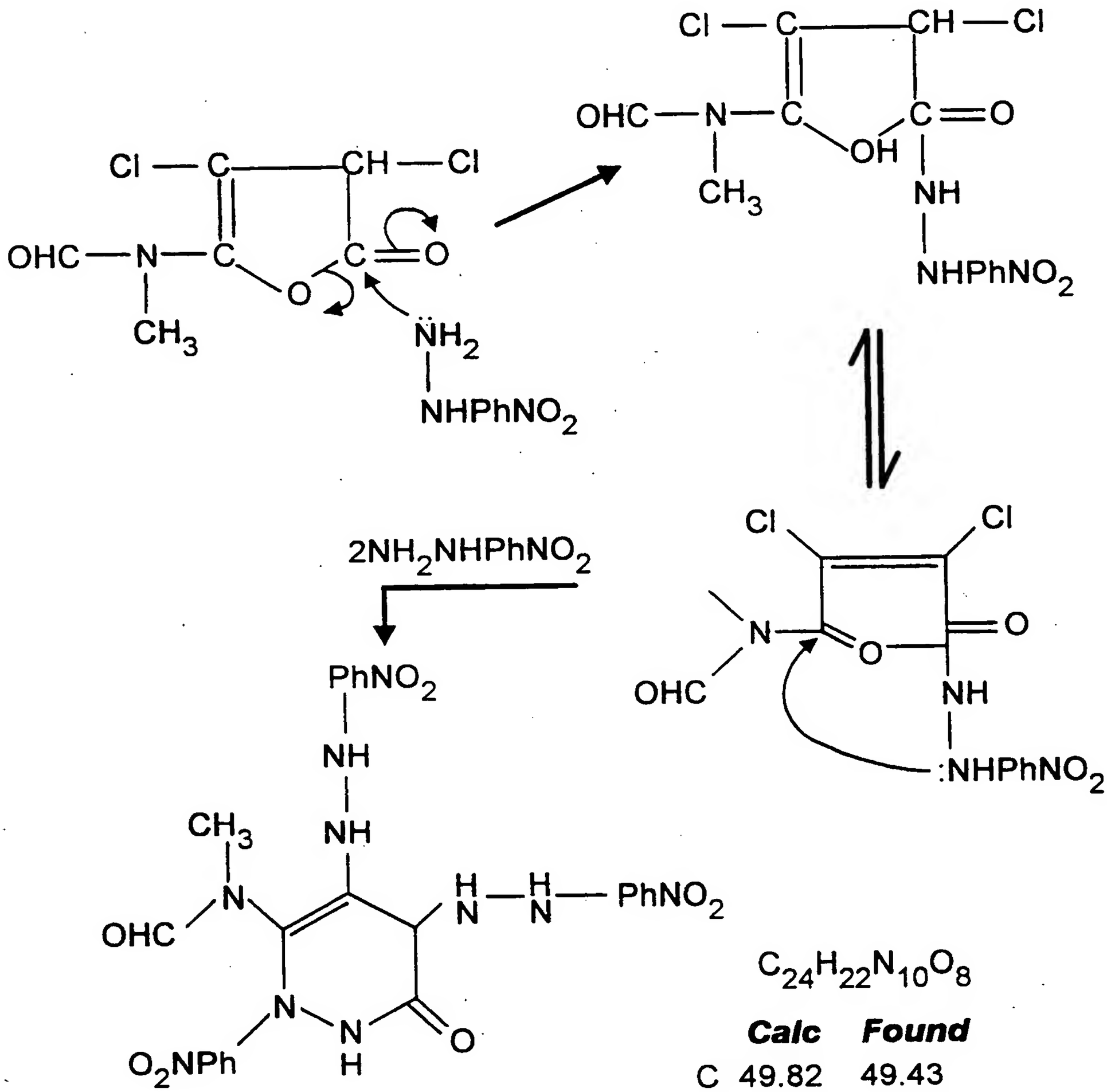


FIG. 23

REACTION OF WOA 081/96B WITH NITROPHENYLHYDRAZINE



$C_{24}H_{22}N_{10}O_8$

	Calc	Found
C	49.82	49.43
H	3.80	3.62
N	24.22	25.00
Cl	0	

FIG.24

Scheme for formation of WOA 081/96B

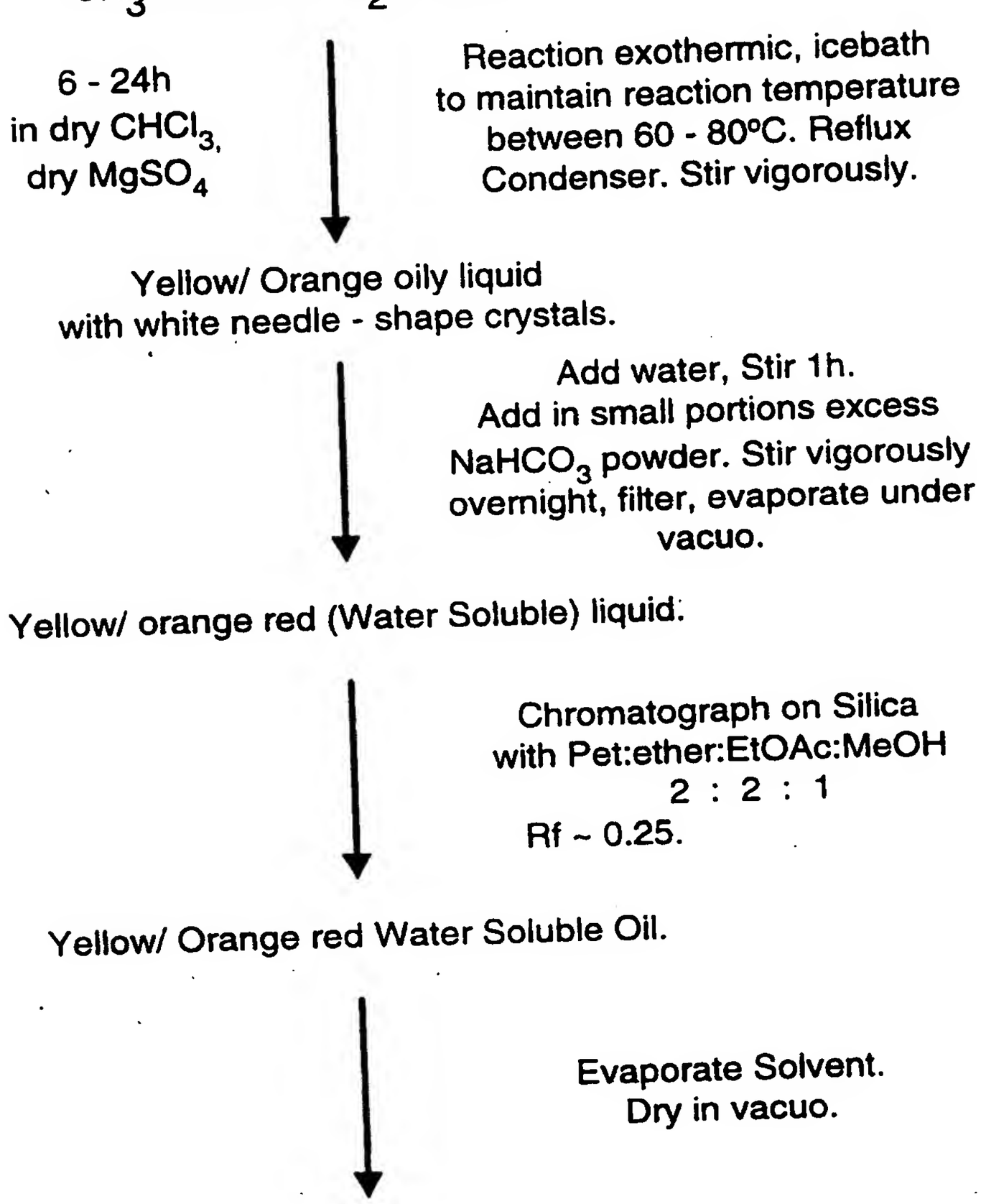


FIG. 25

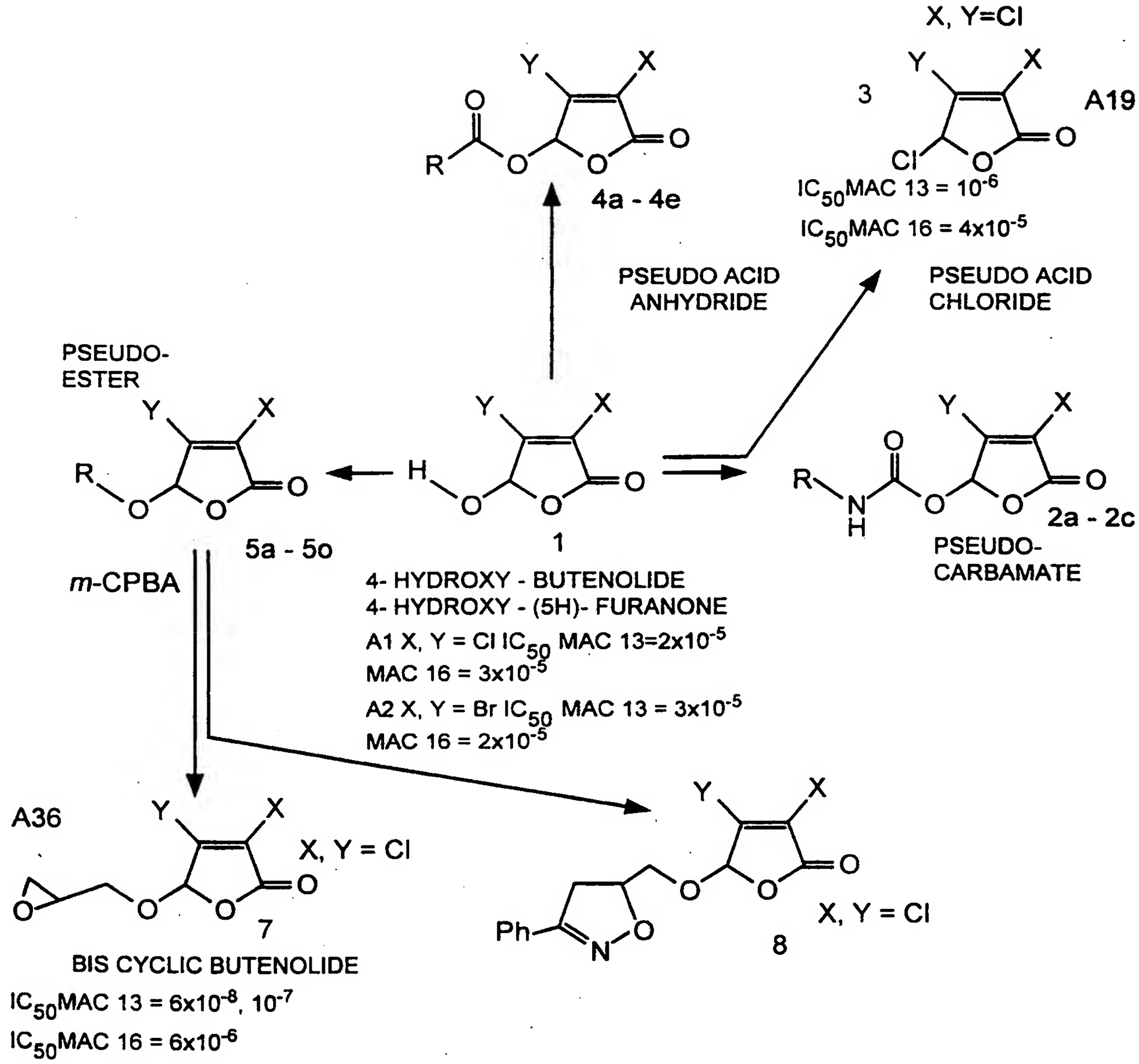

X, Y=Cl
4a - 4e
3
A19
IC50MAC 13 = 10-6
IC50MAC 16 = 4x10-5
PSEUDO ACID ANHYDRIDE
PSEUDO ACID CHLORIDE
PSEUDO-ESTER
5a - 5o
1
2a - 2c
PSEUDO-CARBAMATE
4- HYDROXY - BUTENOLIDE
4- HYDROXY - (5H)- FURANONE
A1 X, Y = Cl IC50 MAC 13=2x10-5
MAC 16 = 3x10-5
A2 X, Y = Br IC50 MAC 13 = 3x10-5
MAC 16 = 2x10-5
m-CPBA
A36
X, Y = Cl
7
BIS CYCLIC BUTENOLIDE
IC50MAC 13 = 6x10-8, 10-7
IC50MAC 16 = 6x10-6
8
X, Y = Cl

FIG.26

THE TABLE BELOW DISPLAYS THE STRUCTURES OF ESTERS SYNTHESISED

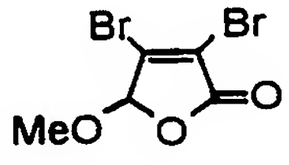
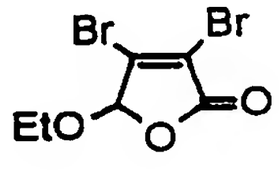
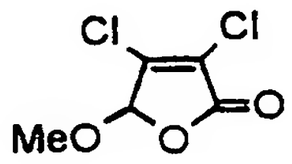
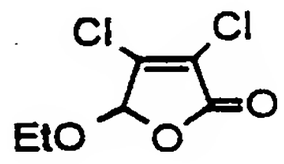
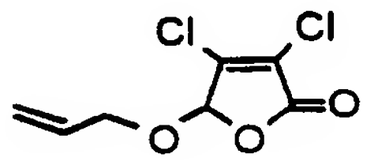
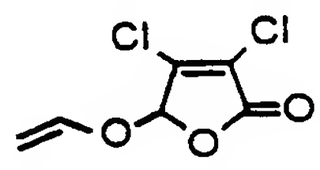
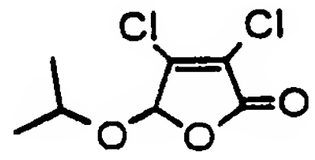
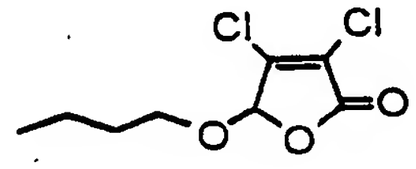
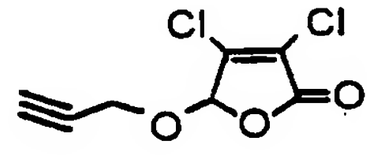
CODE	STRUCTURE	YIELD (%)	IC_{50} MAC 13 / MAC 16	
A9		67	5×10^{-6}	7×10^{-6}
A10		69	10^{-8}	8×10^{-6}
A12		80	6×10^{-6}	7×10^{-7}
A13		84	-	6×10^{-6}
A22		65	8×10^{-6}	5×10^{-5}
A23		59	2×10^{-5}	8×10^{-5}
A25		79	5×10^{-6}	10^{-5}
A26		85	3×10^{-6}	3×10^{-6}
A27		56	2×10^{-6}	3×10^{-6}

FIG.27a

The table below displays the structures of the esters synthesised

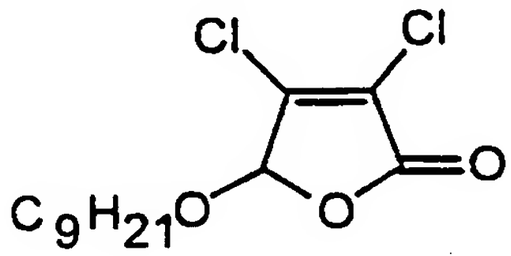
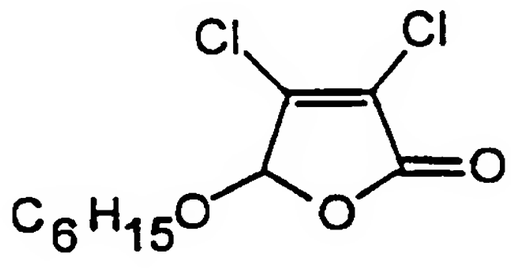
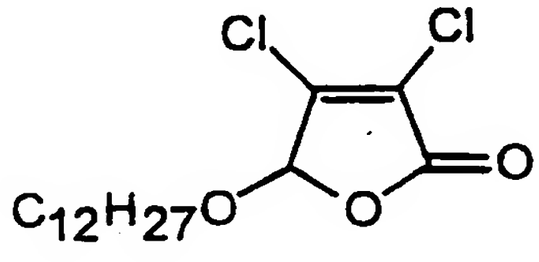
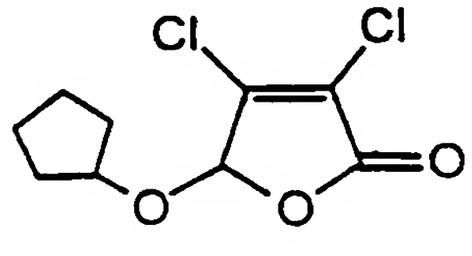
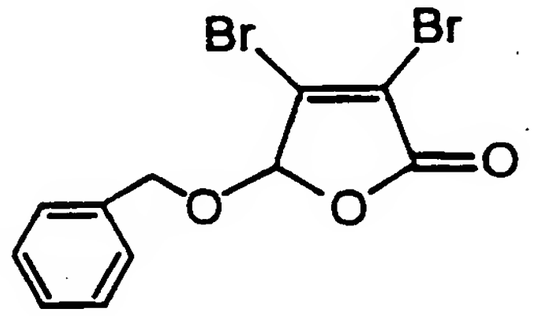
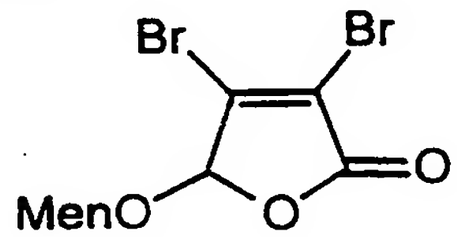
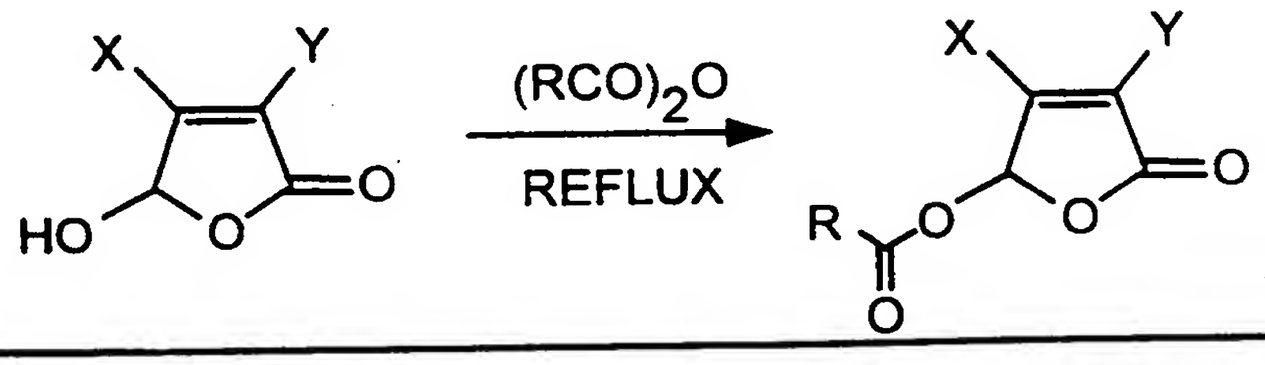
Code	Structure	Yield(%)	IC_{50} MAC 13	IC_{50} MAC16
A51		76	10^{-6}	10^{-5}
A52		82	4×10^{-6}	4×10^{-6}
A53		78	2×10^{-5}	4×10^{-5}
KV4703		—	10^{-5}	3×10^{-5} $>10^{-5}$
KV5001		—	7×10^{-6}	6×10^{-6}
KV5704		—	3×10^{-6}	4×10^{-5}

FIG.27b

MONO AND DIHALOGENATED 5 -ACETOXY - 2, (5H) - FURANONES



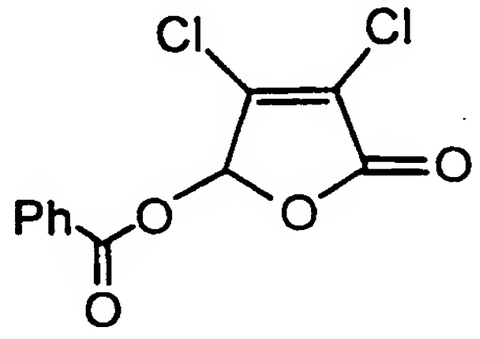
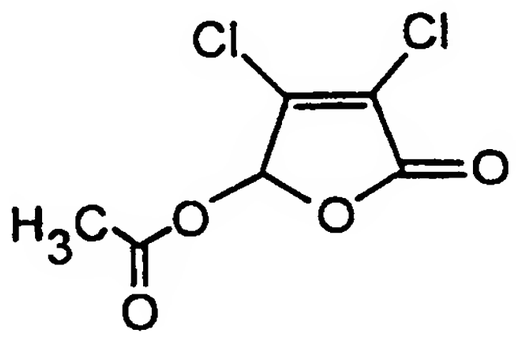
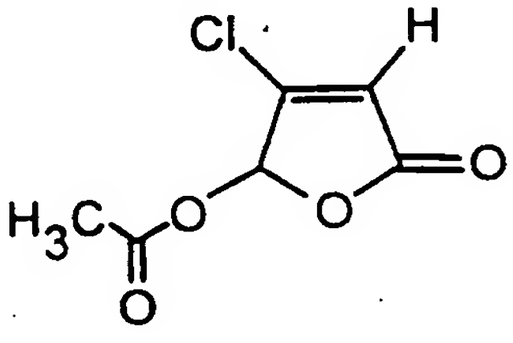
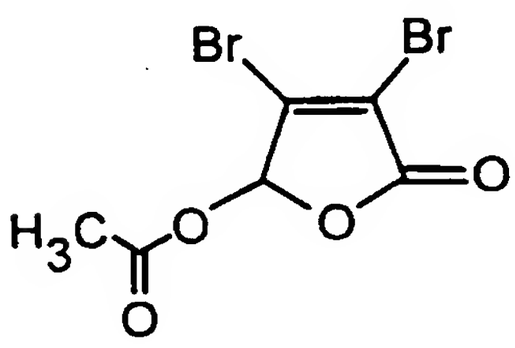
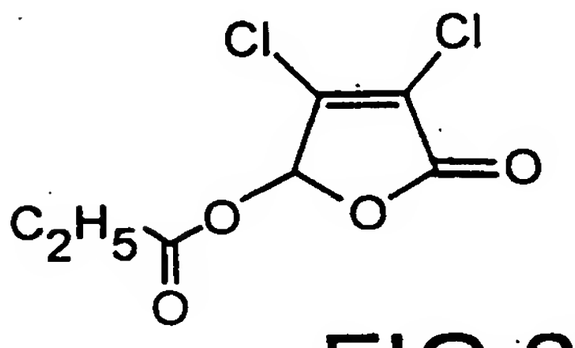
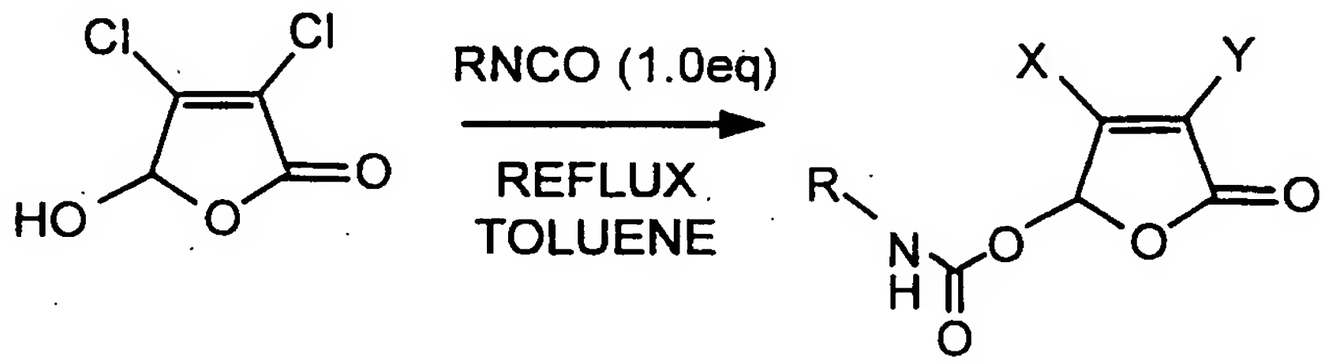
Code	Structure	Yield(%)	IC_{50} MAC 13	IC_{50} MAC16
CL4		79	3×10^{-6}	3×10^{-5}
A32		90	3×10^{-7} 2×10^{-9}	3×10^{-5}
A50		76	3×10^{-5}	7×10^{-5}
A47		81	2×10^{-5}	3×10^{-5}
A54		90	10^{-5}	10^{-5}

FIG.28

REACTION OF MUCOCHLORIC ACID AND CARBANILIC ANHYDRIDES



CARBANILIC ANHYDRIDES

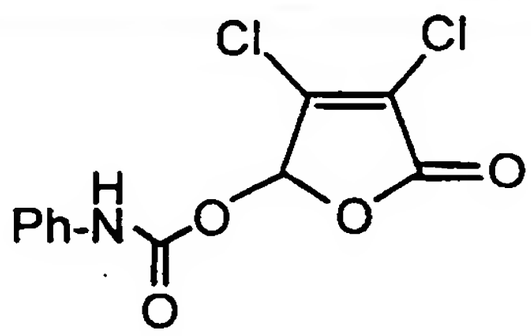
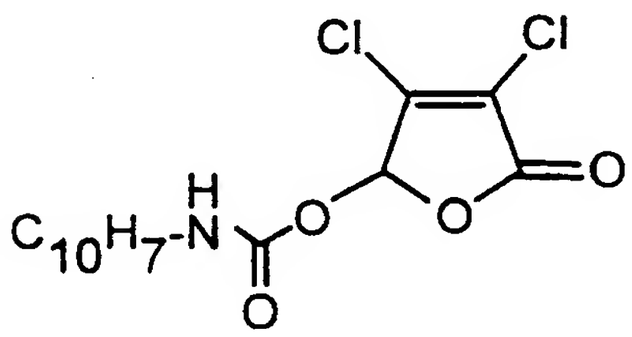
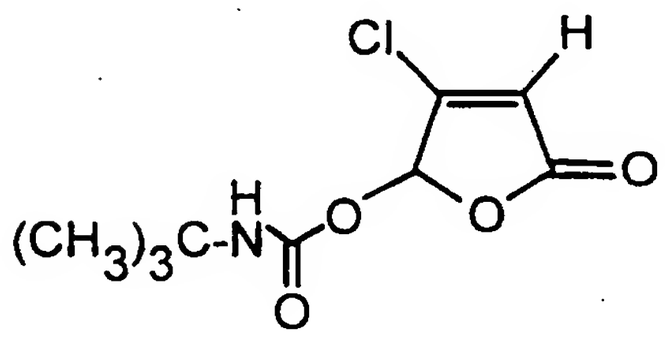
Code	Structure	Yield(%)	IC_{50} MAC 13	IC_{50} MAC16
A28		74	$4x10^{-4}$	$9x10^{-6}$
A55		65	6×10^{-6} 5×10^{-6}	3×10^{-5}
A56		54	4×10^{-6}	2×10^{-5}

FIG.29

SCHEME 1: SYTHESIS OF BISCYCLIC BUTENOLIDES BY USING THE ADDITION OF ACETIC MOLECULES TO THE ALDEHYDE GROUP

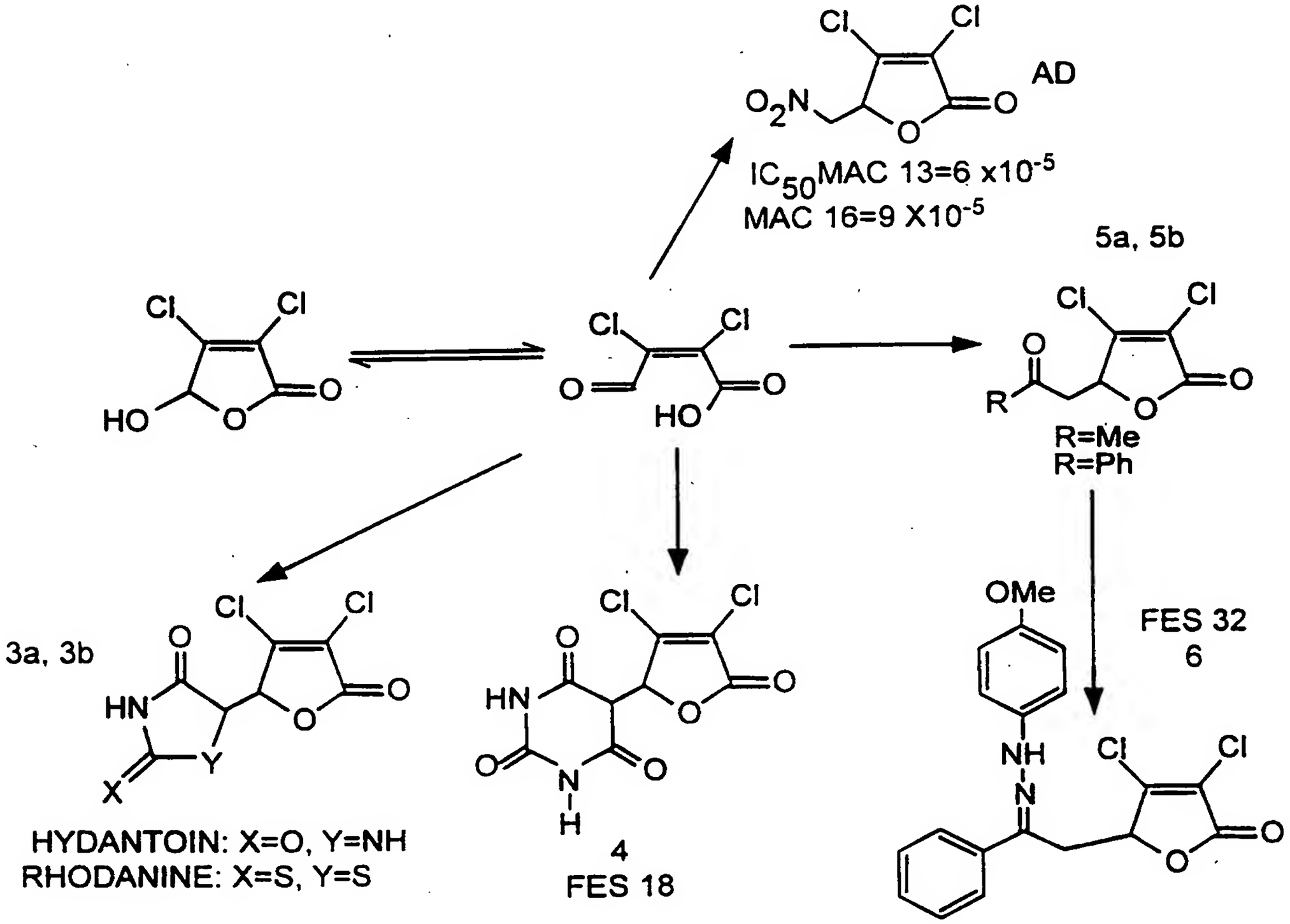


FIG.30

THE TABLE BELOW DISPLAYS THE STRUCTURES OF BICYCLIC COMPOUNDS SYNTHESISED

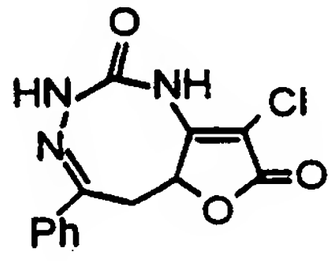
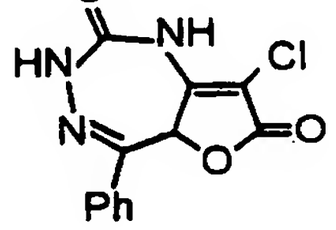
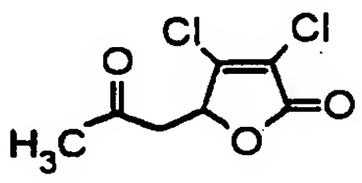
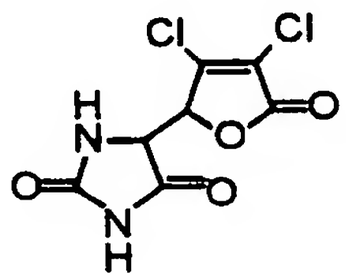
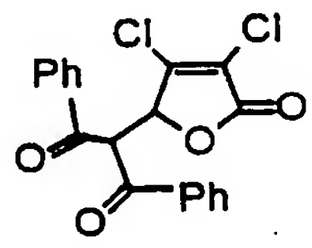
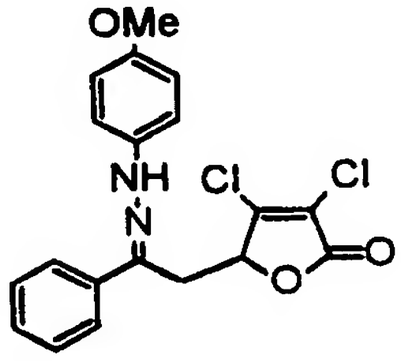
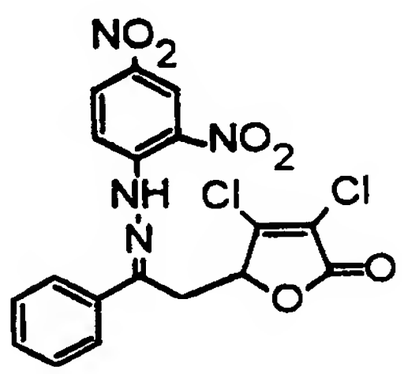
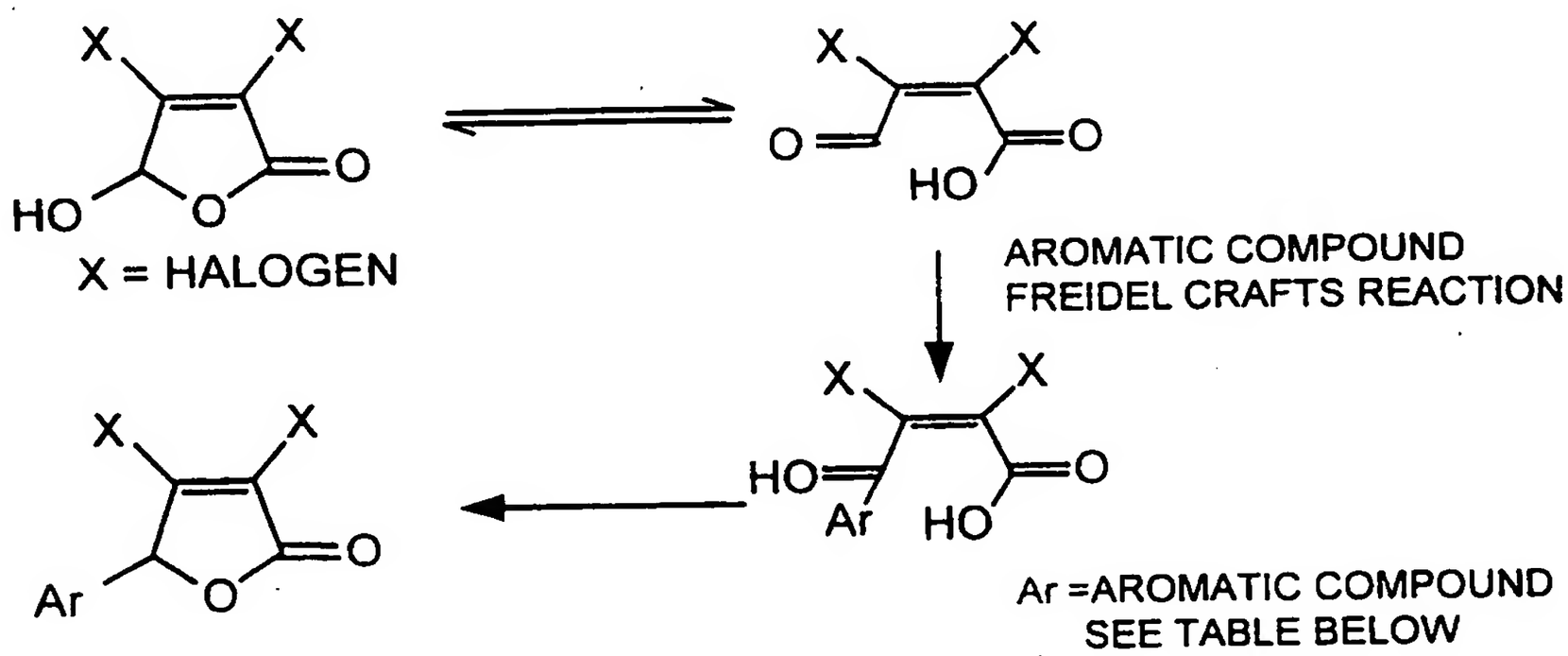
CODE	STRUCTURE	YIELD (%)	IC_{50} MAC 13	IC_{50} MAC 16
FES11		-	3×10^{-5}	3×10^{-5}
FES12		-	7×10^{-5}	2×10^{-5}
FES 15		-	4×10^{-5}	10^{-5}
FES 24		-	2.5×10^{-5}	$>10^{-4}$
FES 25		-	3×10^{-5}	10^{-5}
FES 32		74	1.5×10^{-5}	6×10^{-6}
FES 34		78	-	$>10^{-4}$

FIG.31

SYNTHESIS OF HALOGENATED 5-ARYLATED-2,(5H)-FURANONES



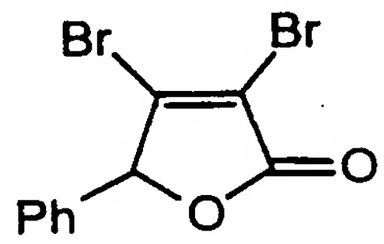
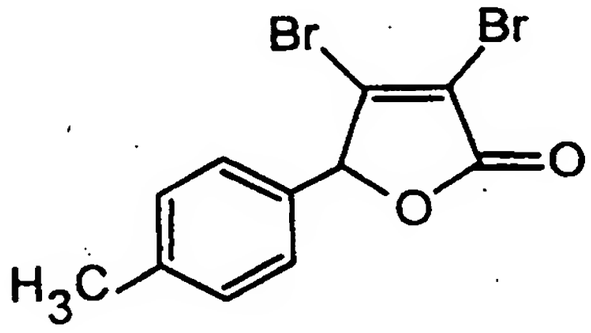
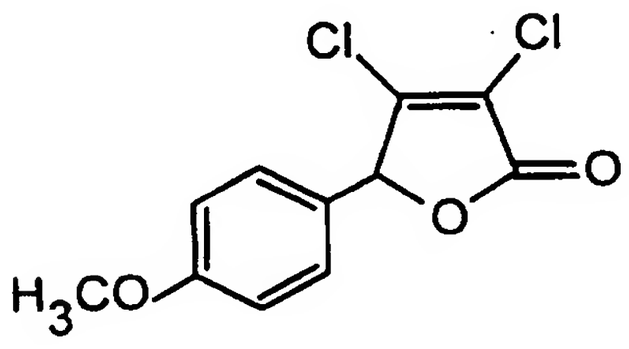
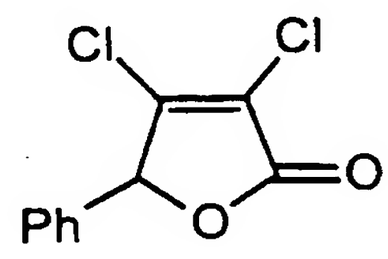
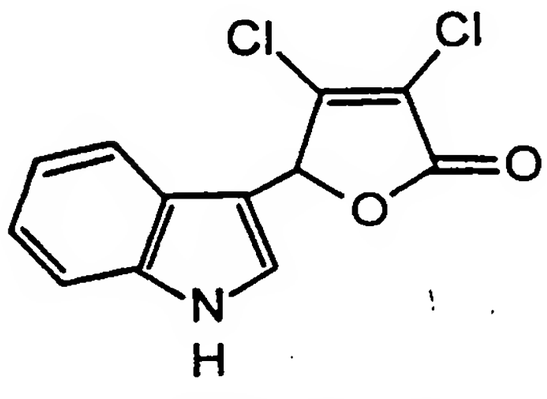
Code	Structure	Yield(%)	IC_{50} MAC 13	IC_{50} MAC16
A41		61	3×10^{-6} 7×10^{-5}	4×10^{-5}
A42		69	-	5×10^{-5}
A43		76	3×10^{-5}	4×10^{-5}
CL1		69	5×10^{-5}	5×10^{-5} 4×10^{-5}
A48		46	3×10^{-5}	7×10^{-5}

FIG.32

THE TABLE BELOW DISPLAYS THE STRUCTURES OF AMINE DERIVATIVES SYNTHESISED FROM ESTERIFIED COMPOUNDS

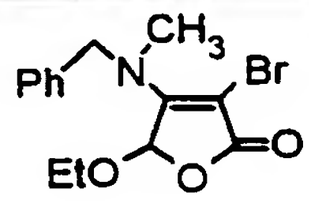
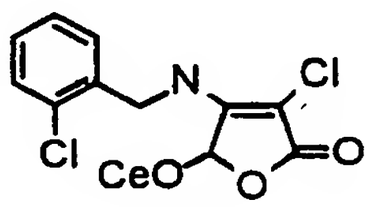
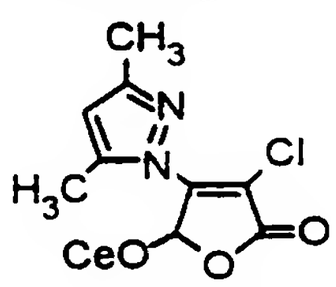
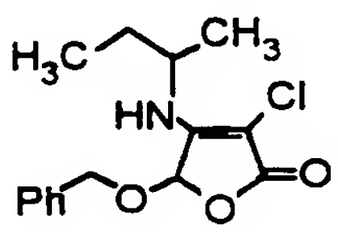
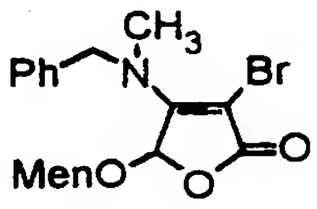
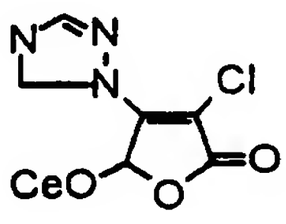
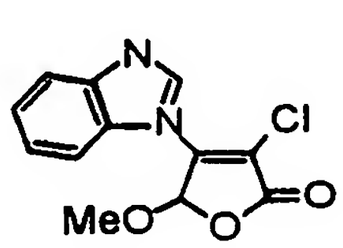
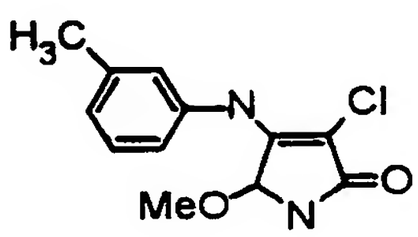
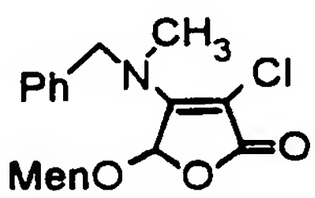
CODE	STRUCTURE	YIELD (%)	IC_{50} MAC 13	IC_{50} MAC 16
A11		68	3×10^{-5}	3×10^{-5}
KV37103B		69	2×10^{-5}	$>10^{-4}$
KV3711		63	$>10^{-4}$	$>10^{-4}$
KV4305BB		-	5×10^{-5}	3×10^{-5}
KV4514		81	6×10^{-6}	10^{-5}
KV3712		72	3×10^{-5}	4×10^{-5}
CV3604B		73	3×10^{-5}	4×10^{-5}
CV4105		-	$>10^{-4}$	$>10^{-4}$
CV4201		65	3×10^{-6}	2×10^{-5}

FIG.33

INTERNATIONAL SEARCH REPORT

Int. ..ational Application No
PCT/GB 99/01074

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D307/58 C07D307/60 C07D307/66 C07D405/04 C07D413/04
C07D491/04 A61K31/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	T. KAWAMORI ET. AL.: "Chemoprevention of azoxymethane induced intestinal carcinogenesis by a novel synthesised retinoidal butenolide, 5-hydroxy-4-(2-phenyl (E)-ethenyl)-2(5H) furanone, in rats." CARCINOGENESIS, vol. 16, no. 4, March 1995 (1995-03), pages 795-800, XP002110157 see whole article ---	1,10,11, 14-16
X	H. HIGASHIDA ET. AL: "Cytotoxic action of retinoidal butenolides on mouse neuroblastoma and rat glioma cells." INTERNATIONAL JOURNAL OF CANCER, vol. 33, no. 5, 15 May 1984 (1984-05-15), pages 677-81, XP002110158 tables I,,II ---	1,10,11, 14-16

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 July 1999	13.08.99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Helps, I

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int...national Application No
PCT/GB 99/01074

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	Y. SATOMI ET. AL.: "Inhibitory effects of (1E,3E,5E,7E)-5-hydroxy-4-(8-phenyl-1,3,5, 7-octatetraenyl)-2(5H) furanone on proliferation of human malignant tumour cells." ANTI-CANCER DRUG DESIGN, vol. 7, 1992, pages 169-79, XP002110159 see whole article	1,10,11, 14-16
Y	US 5 691 373 A (BERRYMAN ET. AL.) 25 November 1997 (1997-11-25) column 19, line 21 - line 34; claims; examples	1-16
Y	WO 97 02265 A (WARNER-LAMBERT) 23 January 1997 (1997-01-23) claims; examples	1-16

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/01074

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: -
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 14 is drawn to a method of treatment of the human or animal body by therapy (Rule 39.1(iV) PCT the search has been carried out based on the alleged effects of the compounds and compositions.

2. [X] Claims Nos.: 1-2, 8-12, 14-16 (partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB 99/01074

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-2, 8-12, 14-16 (partially)

The definition "any functional group" used in claim 1 and dependent claims is non-limiting (Article 6 PCT). The search has been carried out based on the scope covered by the examples (Guidelines B-III, 3.7).

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inc .national Application No
PCT/GB 99/01074

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5691373 A	25-11-1997	AU 693110 B	25-06-1998
		AU 7561794 A	14-03-1995
		CA 2165567 A	23-02-1995
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		EP 0714391 A	05-06-1996
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		NZ 271833 A	22-08-1997
		SK 20396 A	05-03-1997
		WO 9505376 A	23-02-1995
		ZA 9406265 A	19-02-1996
WO 9702265 A	23-01-1997	AU 6388896 A	05-02-1997